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8591 P*

Encephalography with Anesthetic Gases in Man.†

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Anesthetic gases were first used for encephalography by Aird,[1] who reported an experimental series in dogs in February, 1934. He found nitrous oxide and ethylene the most suitable, and felt that a definite anesthesia or sedation was produced in the animals by the injected gas.

On March 6, 1934, we did our first encephalography with nitrous oxide in man. Eight encephalographies were done with this gas, with good radiological results in 3, fair in 4, and poor in one. The subarachnoid channels showed poorly, due to extremely rapid absorption of the subarachnoid gas. The ventricular gas was almost completely absorbed in 24 hours. The immediate reaction to the injection did not differ from that when air is employed, no evidence of anesthesia or sedation being noted. However, post-injection headache was greatly reduced.

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* P represents a preliminary, C a complete manuscript.

† Supported, in part, by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Aird, R. B., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 715.

Subsequent to November, 1934, we substituted ethylene for nitrous oxide, and have done 26 encephalographies with this gas. The reaction of the patients did not differ from that to nitrous oxide, but the radiological results were better, being good in 20 cases, fair in 5, and poor in one. The necessary period of hospitalization after the injection was reduced from an average of 3 days when air was used to 1.85 days with ethylene. Lumbar puncture the following day in 2 cases showed cell counts of 1200 and 1400, mainly lymphocytes, comparable to counts reported after air. In one subject the spinal fluid pressure was followed for 5 hours after the injection, as shown in Figure 1. The negative pressure of 13 cm. of water is due no doubt to the gas, by its relatively great solubility, being absorbed more rapidly than fluid could be formed. The possible consequences of such a marked change in pressure warrant consideration.

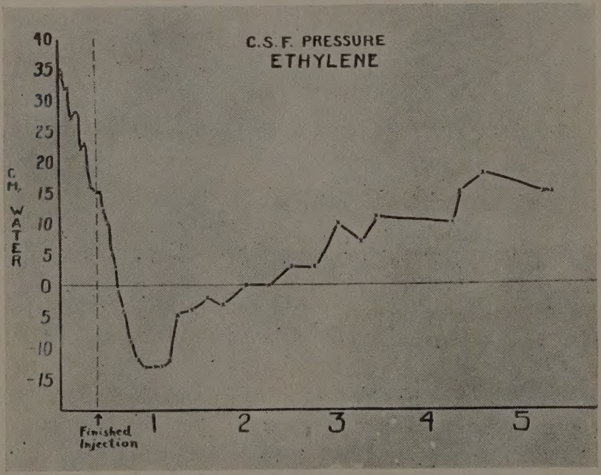

FIG. 1.
Spinal fluid pressure following encephalography with ethylene in man.

8592 P

Oxygen Consumption of Peptone Cultures of *Escherichia coli.*

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Recent studies by Martin,[1] Winslow and co-workers[2] and Clifton[3] on the metabolic activities of bacteria indicate that the rate of metabolic activity per cell varies widely at various phases of the growth cycle. The maximal oxygen-consumption, carbon dioxide-production or ferricyanide-reduction per cell per unit time was noted near the end of the lag-period of growth and could only in part be explained by increased cell-size during the same period of growth. Total oxygen-consumption, carbon dioxide-production or ferricyanide-reduction per unit time reached maximal values near the end of the logarithmic period of growth and then decreased rapidly as the age of the cultures increased.

The present paper reports the influence of peptone- and of oxygen-concentration on the oxygen-consumption, as measured by Warburg's technic, of cultures of *Escherichia coli* at 37.5°C. The oxygen-consumption per cell reached maximal values during the latter part of the lag-period, over a wide concentration range of either peptone or of oxygen. The rate of growth increased slightly as the concentration of peptone was increased from 1.0 to 10% while the total crop was increased approximately 4 times. All cultures were inoculated with the same initial numbers of bacteria. The oxygen-consumption of the cultures varied widely with the age of the cultures and with concentrations of peptone or of oxygen. Typical results are reported in Table I.

The amounts of oxygen consumed per milliliter of the cultures

TABLE I.
Oxygen Consumption of Peptone Cultures of *Esch. coli.*

Age of culture (hours) Medium	2	3	4	5	7	10
	Cu.mm. O_2 consumed/min./ml. of culture					
1.0% peptone (air)	0.65	2.15	2.65	1.20	0.15	0.10
5.0% ” ”	0.72	2.70	6.00	7.15	6.45	2.20
5.0% ” (O_2)	1.34	3.90	11.57	12.60	1.75	1.10
10.0% ” (air)	0.69	2.45	5.80	7.00	7.65	7.00

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[1] Martin, D. S., *J. Gen. Physiol.*, 1932, **15**, 691.

[2] Mooney, Grace, and Winslow, C. E. A., *J. Bact.*, 1935, **30**, 427.

[3] Clifton, C. E., Cleary, J. P., and Beard, P. J., *J. Bact.*, 1934, **28**, 541; Clifton, C. E., and Cleary, J. P., *J. Bact.*, 1934, **28**, 561.

during the first 10 hours were approximately 430 cu. mm. in 1.0% peptone, 2400 cu. mm. in 5% and 3300 cu. mm. in 10% peptone cultures of *Esch. coli.* A marked immediate increase in oxygen-consumption was observed in 24-hour cultures in 1.0% peptone following the addition of small amounts of concentrated solutions of peptone. This suggests that the decrease in metabolic activities observed as the age of a culture increased was due in part to exhaustion of available food. This suggestion is further supported by the observation that no marked growth or oxygen-consumption was observed under the conditions of these tests when filtrates (adjusted to pH 7.2) of 24 hour or older, 1.0% peptone cultures of *Esch. coli* were lightly seeded with this test-organism. Rapid growth and marked oxygen-consumption was noted under the same conditions when 0.1 milliliter of 10% peptone was added per 0.9 milliliter of the filtrates. These results support the previous observations of Clifton[3] on the closely connected rôles played by the concentrations of foodstuffs, oxidant, and organisms in controlling the metabolic activities of bacteria and indicate that these factors must be considered in any interpretation of the different rates of metabolic activity observed during the growth-cycle of bacteria.

8593 P

Calcium Involvement in Magnesium Deficiency.*

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The existence of an intimate relationship between the metabolism of calcium and magnesium in the animal body has long been postulated. In harmony with this hypothesis the writers have accumulated considerable evidence which points to a calcium involvement in the syndrome of magnesium deficiency.

Certain aspects of such an involvement have already been pointed out by Kruse, Schmidt and McCollum[1] and by Orent, Kruse

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* Aided by a grant from The Christine Breon Fund of the Medical School.

[1] Kruse, H. D., Schmidt, M. M., and McCollum, E. V., *J. Biol. Chem.*, 1934, **106**, 553.

and McCollum[2] who observed that animals reared on a magnesium deficient diet showed a marked retention of calcium and an abnormally high percentage content of calcium in their bones.

The phases of the involvement observed by the writers are that there is an accumulation of calcium in the viscera of the body, particularly in the kidneys, and that the magnesium level of the diet at which deficiency symptoms appear is controlled by the dietary level of calcium.

A greater amount of magnesium is required to prevent the development of deficiency symptoms as the calcium of the diet is increased. This is illustrated by the performance of rats reared on diets containing the mineral composition given in Table I.

TABLE I.
Mineral Composition of High and Low Calcium Diets.

	Calcium %	Phosphorus %	Magnesium mg. %	Ca/P
High Calcium diet	1.16	0.62	5	1.85
Low Calcium diet	0.39	0.33	5	1.12

The animals reared on the high calcium diet developed all the features common to magnesium deprivation such as hyperemia, hyperirritability, and cachexia. A large percentage of these animals died from convulsive seizures. Those that survived developed a greasy unkempt appearance, became emaciated, and in other ways showed signs of malnutrition. At the same magnesium level, the rats on the low calcium diet were maintained in a normal state of health and vigor. The females that were bred went through a successful gestation period and gave birth to young of normal weight.

The accumulation of calcium in the viscera of the body of magnesium deficient rats is shown in Table II.

TABLE II.
Effect of Magnesium Deprivation on Magnesium and Calcium Content of Rat Viscera.*

	Control Animals						Deficient Animals					
	Magnesium			Calcium			Magnesium			Calcium		
Tissue	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean	No.	Range	Mean
Heart	8	22.6-29.1	25.7	7	3.6- 6.1	5.15	6	21.2-26.5	23.4	5	6.1- 14.7	8.0
Muscle	17	24.6-32.6	29.4	8	4.8- 7.9	6.35	25	15.2-32.0	25.5	10	8.5- 15.2	10.1
Kidney	17	18.0-25.3	22.2	7	6.9-12.4	9.4	22	16.9-25.3	20.7	10	48 5-232	141

*Analytical figures are in mg. of element per 100 gm. of wet tissue.

From the table it appears that the magnesium content of the soft tissues is not greatly altered by the magnesium deficiency in the diet. The calcium content, on the other hand, is definitely increased. In muscle and heart, the average calcium increase is moderate, being only about 60%. In the kidneys, however, the accumulation is enormous, amounting to a 15 fold average increase. This tremendous increase of calcium in the kidneys probably indicates that metastic calcification forms part of the degenerative process in the kidneys induced by magnesium deprivation. Cramer[3] and the writers have observed the presence of multiple casts, which stain purple with hæmatoxylin and which are probably of a calcareous nature in the kidney sections of deficient rats.

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[2] Orent, E. R., Kruse, H. D., and McCollum, E. V., *J. Biol. Chem.*, 1934, **106**, 573.

8594 C

Protective Action of Specific Serum Against Experimental Vaccinia in Rabbits.

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It is well established that in certain virus diseases such as poliomyelitis immune serum exercises little or no effect on the progress of the infection, even when administered in large doses several days before the usual onset of symptoms.[1] It is also of limited prophylactic value when administered before intranasal instillation with virus.[2] The therapeutic limitations of immune serum in virus diseases generally may be explained by the fact that cytotropic viruses once established within tissue cells are inaccessible to immune bodies in the plasma. Since viruses are primarily intracellular, rather than intercellular parasites, it would appear that the effectiveness of humoral antibodies is likely to be limited largely to infections in which the virus in transported to distant susceptible tissues by the blood stream, or tissue fluids. In diseases such as poliomyelitis, in which the virus is nerve-transmitted throughout and may gain admission to susceptible tissue without necessarily passing a barrier of immune plasma[3] the prospects of establishing significant protection against infection by the natural or intranasal route appear little better than those of gaining a therapeutic effect once infection is established. This is borne out by the observations already mentioned.[1, 2] In this paper we deal with a virus (vaccinia*) which although "neurotropic" does not, like poliomyelitis virus, depend on intact nerve paths for its transmission to susceptible cells, but may be disseminated by the blood stream and lead to generalized infection. More specifically, these studies are concerned with the protective action of immune serum in rabbits inoculated with vaccinia virus by the intranasal route.

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[3] Cramer, W., *Lancet*, 1932, **223**, 174.

[1] Schultz, E. W., and Gebhardt, L. P., *J. Pediat.*, 1935, **6**, 615.

[2] Schultz, E. W., and Gebhardt, L. P., *J. Pediat.*, 1935, **7**, 332.

[3] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.

Halva and Honl[4] 40 years ago reported that serum from immune calves when injected subcutaneously into normal calves conveys some degree of protection against subsequent cutaneous inoculation with vaccinia virus. Since then a number of investigators have reported experimental observations which show quite clearly that immune serum may convey well defined protection against subsequent inoculation with virus.[5] Observations on the protective value of immune serum against intranasally instilled vaccinia virus have apparently not been reported thus far.

Pools of immune serum were prepared from the blood of 11 rabbits hyperimmunized by repeated subcutaneous injection of vaccinia virus (Gallardo strain) following recovery from cutaneous vaccinia. Titration of these pools showed that 1 cc. of serum contained sufficient antibodies to neutralize at least 16,000 minimum skin infecting doses. Preliminary observations on the effectiveness of intranasal instillation as a means of infecting normal rabbits indicated that as little as 10 minimum skin infecting doses sufficed to establish generalized vaccinia in 5 to 6 days. The technic of inoculation was essentially that described by Schultz and Gebhardt[6] for the intranasal inoculation of monkeys with poliomyelitis virus. The total amount of virus instilled intranasally into each animal amounted to about 10,000 minimum skin infecting doses. The immune serum was administered intravenously in all cases. One rabbit received 3 daily injections of 5 cc. each 3, 2 and one day preceding inoculation with virus; 2 rabbits received 2 injections of 5 cc. each, 2 days and again 1 day preceding the inoculation. Eight rabbits received a single injection of 2 cc. per kilo of serum 1, 2, 3, 4, 7, 14, 21, and 35 days, respectively, preceding inoculation. All but one of the animals receiving serum were protected. This animal had received the serum 35 days previously. All of the controls (4 in number) developed generalized vaccinia in 6 to 7 days.

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* Gallardo strain.

[4] Halva, J., and Honl, I., *Wein. klin. Rundschau*, 1895, **9**, 625, 643.

[5] Béclére, A., Chambon and Ménard, *Ann. L'Inst. Past.*, 1899, **13**, 81; Camus, L., *Compt. rend. Soc. Biol.*, 1912, **73**, 197, 294; Haendel, L., Gildemeister, E., and Schmidt, H., *Centralbl. f. Bakt., Abt. I., O.*, 1920, **85**, Beiheft, 125; Hunt, L. M., and Falk, I. S., *J. Immunol.*, 1927, **14**, 347; Andrewes, C. A., *J. Path. and Bact.*, 1929, **32**, 265; Craigie, J., and Tulloch, W. J., *Med. Res. Council, Special Rept. Series*, No. 156, 1931.

[6] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **33**, 1010.

Another series of animals were given serum injections at varying intervals of time *following* intranasal instillations of virus. Five rabbits received 4 cc. of serum per kilo of animal weight 1, 6, 12, 24 and 36 hours, respectively, after inoculation. All were protected, while the controls developed generalized vaccinia on the 6th day. Four additional rabbits received the same dose of serum 24, 48, 72 and 96 hours, respectively, following inoculation. The 2 animals which received the serum within 2 days survived, while the 2 which received the serum later developed generalized vaccinia on the 6th day.

The results suggest that passive immunization is considerably more effective in protecting rabbits against generalized vaccinia following intranasal instillation of virus than in protecting monkeys against intranasal inoculation with poliomyelitis virus. This harmonizes with evidence already available[3] that in poliomyelitis, the virus is transmitted from the olfactory mucosa to the central nervous system by the olfactory nerves essentially out of the reach of immune substances in the blood plasma.

8595 C

Dietary Production of Specific Syndrome of Deficiency in Vitamin B_6.*†

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As a result of recently reported work from many laboratories it appears certain that the factor formerly known as vitamin G

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† Presented before the 91st meeting of American Chemical Society in Kansas City.

(B_2) is a complex—one member of which is flavin and the other a relatively heat- and alkali-stable factor. Lack of this second factor leads to a "specific" form of dermatitis described in detail by Chick and co-workers,[1] György[2] and others.

Some difficulty, however, has been noted in various laboratories in inducing this particular dermatitis. The Sherman-Bourquin diet[3] has been found very successful in general, particularly when the added precaution has been taken of using the Page anticoprophagy harness.[4] However, György noted that different results were obtained when different lots of wheat were used. Hogan and Richardson[5] have shown that cornstarch contains an alcohol-soluble substance which cured dermatitis in their rats when they were given a diet of casein, sucrose, salts, cod liver oil and cellulose, plus an irradiated aqueous extract of yeast. If the diet contained starch in place of sucrose no dermatitis developed. The condition was also cured by wheat germ oil.

One of us has used the Sherman-Bourquin diet over a period of some years and has not had difficulty in inducing the dermatitis and cessation of growth. However, several attempts to repeat this in this laboratory have proved unsuccessful, even when harnesses were used. The rats when receiving no additional supplement to the basal diet ("negative control rats") continued to grow and showed no skin abnormalities. It was then suspected that the wheat was unusually rich in the B_2 factors and that they were extracted along with vitamin B_1 by the alcohol. We were fortunate in receiving from Merck and Company some vitamin B_1 crystals as well as a highly potent concentrate from rice polish. When the Sherman-Spohn diet[6] (deficient in all known B factors) was substituted, plus the B_1 concentrate, the incidence of dermatitis was greater. Practically all animals developed skin lesions after receiving the diet for 6 to 8 weeks.

Booher and co-workers[7] have shown that the flavin component of vitamin B_2 is extracted from whey by hot 95% alcohol. When casein is extracted with hot concentrated alcohol (as in the Sherman-Munsell[8] method for its preparation for use in vitamin A-free diets) the resultant alcohol is always fluorescent. The usual method for extraction of casein for the Sherman-Spohn diet is by the use of cold 60% alcohol. We felt that, due to the extractability of the fluorescent material with hot concentrated alcohol, such treatment might be advisable. We therefore adopted the method of Sherman and Munsell and followed this by 2 cold 60% alcohol extractions, with quite satisfactory results.

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[1] Chick, H., Copping, A. M., and Edgar, C. E., *Biochem. J.*, 1935, **29**, 722.

[2] György, P., *Biochem. J.*, 1935, **29**, 741.

[3] Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 3501.

[4] Page, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 87.

[5] Hogan, A. G., and Richardson, L. R., *J. Nut.*, 1935, **8**, 385; *Nature*, 1935, **136**, 186; *Science*, 1936, **83**, 17.

[6] Sherman, H. C., and Spohn, A., *J. Am. Chem. Soc.*, 1923, **48**, 2791.

[7] Booher, L. E., *J. Biol. Chem.*, 1932, **102**, 39; Booher, L. E., Blodgett, H. M., and Page, J. W., *J. Biol. Chem.*, 1934, **107**, 599.

Young rats at 21 days of age weighing about 45 gm., when given this modified Sherman-Spohn diet plus a vitamin B_1 concentrate (in amounts which induce a gain of 8 to 10 gm. weekly in rats receiving a vitamin B_1 deficient diet) gained 10 to 15 gm. in about 2 weeks. By the end of this period about 35% of the rats showed rough paws with inflammation between the toes. These symptoms became progressively worse. In order to compare the results obtained with this diet with results from other laboratories, it was necessary to feed the deficient rats supplements similar to, if not identical with, the materials used by other workers. To this end we prepared alcoholic and aqueous extracts of brewer's yeast and aqueous extracts of fresh hog liver. These were treated with Fuller's earth according to the methods used in the preparation of flavin by other investigators. The adsorbates and resulting filtrates were fed separately or in combination. Results from these experiments will be reported in detail at a later date. However, of 35 rats receiving no supplement (except vitamin B_1), or receiving the material adsorbed by Fuller's earth from the yeast or liver extracts, 30 showed raw and edematous paws, swollen ears and sores around the nose and mouth by the end of 5 weeks. There was no alopecia. Death has occurred in some cases as early as the fourth week. Care was taken to prevent access to feces, but the harnesses were not used. In confirmation of Chick and coworkers it seemed that feeding of the adsorbed material (the flavin component) intensified the symptoms. The filtrates from the yeast extract, even after autoclaving for 1 hour at pH 9, prevented the lesions. The liver filtrate was effective in both curative and prophylactic experiments.

In all of this work cornstarch was used. In order to determine whether the use of sucrose would be more satisfactory, a diet was made up in which sucrose was substituted for the starch. The diet contained 8% butter fat and 2% cod liver oil, and these do not blend with sugar as with starch. The animals became very greasy in appearance and there was some slight alopecia, probably due to irritation. The "specific" skin symptoms, however, did not appear earlier and were no more severe. Death occurred somewhat earlier in the case of the negative control rat, and a greater length of time was required to induce a cure.

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[8] **Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, 47, 1639.**

Itter and co-workers[9] have correlated alopecia with the absence of the sulfhydryl group and found that feeding of *cysteine*-HCl led to cures. Prunty and Roscoe[10] showed that purified casein might be deficient in cystine, with the result that the growth rate was lessened. They could not cure the "specific" dermatitis with cystine, however. Whether or not our treatment of casein would lower the cystine content to such an extent as to be a factor in our results is being investigated.

It may be concluded that the "specific" dermatitis and cessation of growth can be readily induced in rats receiving a diet containing cornstarch, provided a highly purified and potent vitamin B_1 concentrate is used and the casein is so extracted as to remove the B_2 factors. Whether this dermatitis is identical with that induced by Hogan and Richardson remains to be determined.

8596 C

Veterinary Staphylo-Fibrinolysin.*

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In an examination of 132 local strains of staphylococci† it was found that 80% of all strains originally isolated from internal human lesions are capable of liquefying human fibrin.[1] Approximately 90% of all strains isolated from superficial human infec-

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[9] Itter, S., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1935, **108**, 585.

[10] Prunty, F. T. G., and Roscoe, M. H., *Biochem. J.*, 1935, **29**, 2491.

* Work supported in part by the Eli Lilly and Co. Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

† The strains and clinical histories used in these surveys were kindly furnished by the various hospitals, clinics, diagnostic laboratories, and veterinary institutions of the San Francisco Bay Region.

[1] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 209.

tions, however, and all strains isolated from veterinary lesions are non-lytic by the same *in vitro* technic.

We have retested these and 9 additional local strains of *Staphylococcus aureus* or *albus* with fibrins isolated from lower animals. Data thus obtained are summarized in Table I.

TABLE I.

Effect of Staphylo-lysins on Lower Animal Fibrins.

24-hour veal-infusion broth cultures *of Staphylococcus aureus or albus* tested against serum-free fibrins by the method of Tillett and Garner.[2] +++, complete liquefaction of the fibrinogen-thrombin complex within 30 minutes. ++, complete liquefaction within 2 hours. +, partial liquefaction by the end of 2 hours. 0, no demonstrable softening of the fibrin-clot by the end of 2 hours.

	Lysis of serum-free fibrin-clot from:					
No. of strains	Monkey	Man	Rabbit	Horse	Sheep	Other animals*
Group A (multivalent)						
1 strain	+++	++	++	0	++	0
12 "	+++	++	++	0	0	0
1 "	+++	+	+	0	0	0
4 "	++	++	++	0	0	0
Group B (bivalent)						
1 strain	+++	0	++	0	0	0
1 "	+++	+	0	0	0	0
7 "	++	++	0	0	0	0
1 "	++	0	+	0	0	0
1 "	0	++	++	0	0	0
Group C (univalent)						
11 strains	+++	0	0	0	0	0
19 "	++	0	0	0	0	0
1 "	+	0	0	0	0	0
1 "	0	++	0	0	0	0
1 "	0	+	0	0	0	0
2 "	0	0	0	++	0	0
Group D						
77 strains	0	0	0	0	0	0

*Rat, hog, cow, domestic fowl.

The table suggests that there are at least 5 different fibrinolytic factors formed by pathogenic staphylococci. Each lytic factor is apparently specific for the fibrin of one animal species. At least 3 of these species-specific fibrinolysins are apparently independent variables in staphylococci.

Of particular interest are the 2 monovalent horse-lytic strains (Group C). These 2 strains were originally isolated by the Cutter Laboratory from "abscesses" in horses. With these 2 exceptions all fibrinolytic staphylococci thus far tested by us were of human origin, all other local veterinary strains being included in Group D.

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[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

8597 C

Fibrinolytic Specificity of *B. pestis*.*

R. R. Madison. (Introduced by W. H. Manwaring.)

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Applying the plasma-clot technic, Tillett and Garner[1] demonstrated fibrinolytic enzymes in broth cultures of streptococci and staphylococci. All other bacterial species tested by them were fibrinolytically negative.

A negative reaction with the plasma-clot technic, however, is not conclusive evidence of the absence of a fibrinolytic factor. Many plasma-clots are known to contain neutralizing antibodies. We

TABLE I.

Rate of *B. pestis* fibrinolysis.

0.5 cc. 24-hour veal-infusion broth culture of *B. pestis* plus 1 cc. dilute fibrinogen-thrombin complex, prepared by the technic of Tillett and Garner.[1] ++++, complete lysis of the resulting fibrin-clot within 15 minutes; +++, within 30 minutes; ++, 60 minutes; +, 2 hours; ±, partial liquefaction, 2 hours; 0, no demonstrable softening of the clot by the end of 2 hours incubation. (Note: With some of the apparently insusceptible fibrins, partial or complete lysis was demonstrable after 4-6 hours incubation.)

B. pestis	Lysis of serum-free fibrin isolated from:								
strain No.	Man	Rhesus monkey	Ground squirrel	Guinea pig	Rat	Rabbit	Cat	Cow	Other animals*
1	+	0	+	±	±	0	+	±	0
2	+++	0	+	+++	++	++	++	+	0
3	+++	0	+	++++	+++	++	+	+	0
4	++	0	++	++	++	++	+	++	0
5	+++	0	++	+++	+++	++	++	+	0
6	++	+	+	++++	+++	++	++	+	0
7	++	+	+	+++	+++	+++	++	+	0
8	+++	+	++	+++	+++	++	++	+	0
9	+++	++	+	++++	++++	+++	++	++	0
10	+++	+	±	++++	+++	+++	±	+	0
11	+++	+	++	+++	+++	+++	++	++	0
12	+++	0	±	++	+++	++	±	0	0
13	+++	+	+	+++	+++	+++	++	+	0
14	+++	0	+	+++	++	++	+	+	0
15	++	+	±	++++	+++	++	++	0	0
16	+++	+	++	+++±	+++	+++	++	+	0
Control (autolysis)	0	0	0	0	0	0	0	0	0

*Sheep, horse, hog, hen.

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* Work supported in part by the Eli Lilly and Co. Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

have, therefore, tested all locally available bacterial species (231 strains) by the serum-free fibrin-clot technic.

In addition to certain pyogenic cocci, all locally available strains of *B. pestis* are strongly fibrinolytic, particularly when tested with rat- or guinea pig-fibrin. Fibrinolytic tests with the 16 available bubonic plague cultures are recorded in Tables I and II.

TABLE II.

Fibrinolytic Titer of *B. pestis* cultures.

Serial dilutions (1:2) of 24-hour veal-infusion broth cultures of *B. pestis* tested against serum-free fibrin-clots (*cf.* Table I). The highest dilution of the culture giving complete liquefaction of the fibrin-clot by the end of 2 hours was assumed to contain one arbitrary fibrinolytic unit.

B. pestis strain No.	Lytic units per 0.5 cc. when tested against fibrin from: Man	Rhesus monkey	Ground squirrel	Guinea pig	Rat	Rabbit	Cat	Cow	Other animals*
1	8	0	8	1	2	0	2	1	0
2	32	0	8	28	256	4	8	2	0
3	32	0	8	32	256	4	2	2	0
4	16	0	24	8	64	4	2	3	0
5	32	0	24	28	256	4	16	2	0
6	16	2	8	32	64	4	16	2	0
7	16	2	8	28	64	4	8	2	0
8	32	2	8	28	64	4	16	2	0
9	32	3	8	32	256	4	8	3	0
10	32	2	2	32	256	4	1	2	0
11	32	2	24	28	256	4	16	3	0
12	28	0	2	8	64	4	2	0	0
13	32	2	8	28	256	4	8	2	0
14	32	0	8	28	256	4	8	2	0
15	16	2	8	32	64	4	2	2	0
16	32	2	24	32	256	4	16	2	0
Average 2-16	28	1	11	27	179	4	9	2	0
Relative susceptibility of fibrin	16%	½%	6%	15%	**100%**	2%	5%	1%	0%

*Sheep, horse, hog, hen.

Strain 1 in Tables I and II is a 20-year-old stock culture of *B. pestis* originally isolated from a human case. Strains 2-16 have been isolated during the last 12 months from field mice and ground squirrels. These 15 strains were kindly furnished for our work by Dr. Karl F. Meyer, Hooper Foundation, University of California.

Table II shows that the fibrinolytic factors formed or secreted by the 15 rodent strains are relatively specific (or of relatively high titer) for rat-fibrin. The one available human strain, however, yields lytic factors of relatively high titer for ground squirrel and human fibrins.

Southern Section

Tulane University, February 26, 1936.

8598 C

Biological Study of "R" and "S" Forms of Chromogenic Acid-Fast Bacillus from Human Leprous Lesion.

J. R. KRIZ. (Introduced by C. W. Duval.)

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A great deal has been written concerning the various strains of bacilli isolated from leprous patients. Of the many strains, 2 or 3 have most consistently been isolated by investigators. The 3 most commonly cultivated from the leprous lesion are: the organism described by Clegg,[1] which is an acid-fast chromogenic bacillus; Kedrowski's[2] non-acid-fast diphtheroid; and a bacillus isolated by Duval[3] which is permanently acid-fast and maintains *in vitro* the tinctorial and cultural characteristics of the Hansen bacillus of the tissues. At the present time, however, it is almost universally believed that an acid-fast bacillus, which is found in the leprous nodule, is responsible for leprosy.

In spite of the large amount of research on the artificial cultivation of the leprosy bacillus and the variety of forms exhibited by this bacillus comparatively little concerns rough and smooth forms.[4] With this in mind attempts were made to isolate these variants and study them biologically with the purpose of determining cultural and morphological differences, rate of growth and eventually any difference in pathogenicity.

An acid-fast bacillus isolated from a subcutaneous leprous nodule and cultivated according to the method of Duval[5] grew readily upon the ordinary nutrient media, having successfully passed through the transitional period of *in vitro* adaptation. The medium used was glycerol-agar. The Hansen bacillus, after its adaptation to artificial cultivation, is a gram-positive acid-fast rod. The bacilli varied in size and shape, some were short and plump with rounded ends while others were somewhat slender and slightly curved. The distribution of the chromatin varied; some bacilli showed uniform staining, others large bipolar granules, still others small granules or beading, numbering from 2 to 5 and distributed evenly along the entire cell. Growth was visible after 3-4 days at 37.5° and resembled that of the tubercle bacillus in that it was irregular and dull yellow with a wrinkled, dry surface. During its adaptation on special media, however, the growth was moist, pale yellow with a smooth, glistening surface.

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[1] Clegg, M. T., *Philippine J. Sc.*, 1909, **4**, 77; *ibid.*, 403.

[2] Kedrowski, W., *Z. f. Hyg. u. Infektionkr.*, 1901, **37**, 52.

[3] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

[4] Salle, A. J., *J. Infect. Dis.*, 1934, **54**, 347.

[5] Duval, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 498.

A wrinkled culture was streaked several times on glycerol-agar and within 3-4 days many isolated colonies were visible. These were transferred to other plates and subcultured. All colonies were flat with an irregular outline and a dull, wrinkled surface. After 6 subculturings, all colonies had the same characteristics already described. When incubated 6-7 days, there appeared very small, convex, moist colonies somewhat clear and honey-like in color and consistency. These were seen in and about the wrinkled colonies. Several that were separated from the rough colonies were carefully transplanted to glycerol-agar plates and incubated at 37.5° for 6-7 days. Except for a few rough colonies the entire plate showed the same characteristics of the smooth form. Several isolated colonies were again transferred to fresh medium and a pure culture of the "S" form was obtained. An isolated rough colony was carefully replated several times and after incubation for several days no smooth colonies could be seen. These variants remained colonially pure. The "R" form grew more rapidly than the "S."

Tinctorially, the majority of the "S" bacilli were gram-positive slender rods accompanied by a few pale-staining smaller bacilli which appeared gram-negative. Some were slightly curved, others contained small granules varying in number from 2-5 in a cell, still others showed only bipolar staining. The "R" form showed a predominance of gram-positive, short, plump rods. No small granules could be seen. Either the rods were solidly stained or only bipolar staining was present. The bipolar staining was not like that seen in the "S" form. Here the chromatin extended almost to the center of the cell, diplo-bacillary in form, leaving only a faint separation between the 2 large granules. If the light is cut down, there can be seen about most of the bacilli a definite clear zone not unlike a capsule. While this was present in the "S" form, the halo in the "R" form was definitely much thicker and more conspicuous. With the Ziehl-Neelsen stain the "S" form showed about equal numbers of acid-fast and non-acid-fast rods. The acid-fast rods were slender bacilli, some contained 2-5 small granules others had small bipolar granules while some were uniformly stained. The non-acid-fast rods appeared shorter and stained uniformly. The "R" form showed a predominance of short, plump, acid-fast rods which had large bipolar granules. No small granules were seen in the "R" form. Loeffler's methylene blue rendered the halo about the "R" form more clear and prominent.

Culturally the "R" form is a spreading, flat irregular colony with a dry, wrinkled surface. There is a fairly good growth after 4 days. The "S" form grows more slowly. In about 6-7 days a small circular, convex colony with a smooth, moist, surface becomes visible. If the cultures are allowed to stand at room-temperature for 2 weeks, the growth, especially of the "R" form becomes a deeper yellow. Saline suspensions of the "R" form are granular and flaky, the growth adhering to the sides of the tube; the "S" form yields a uniformly turbid suspension. Both types were subjected to 53°C. for 5 and then for 10 minutes, repeating for every 5 degrees up to 73°. The "R" survived a temperature of 68° after 5 and 10 minutes while the "S" showed presence of growth on glycerol agar plate up to 63°.

Summary. The "R" and "S" forms of the lepra bacillus show definite biological differences. The "S" form is a smooth and moist colony while the "R" form is wrinkled and dry. The "R" form grows the more rapidly, forms a flaky suspension and is more thermostable. After several months of subculturing, both forms remained pure. The "S" form are acid-fast rods the majority of which contain 2-5 small granules. The "R" form are short rods showing a predominance of large bipolar granules and surrounded by a thicker capsular zone. The significance of the small granules in the "S" form and the large polar granules in the "R" form is not fully understood. It is reasonable to believe that the small granules in the "S" form are signs of degeneration while the large granules in the "R" form may represent a stage of quiescence resembling a spore stage. The presence of the much thickened capsular zone and the higher thermal death-point in the "R" form may have some relation to its stability and resistance to disintegration.

Since the "S" form reverts to the "R" during its adaptation on artificial media and the "S" variant can later be isolated from the "R" it must be assumed the "S" and "R" are reversible. The "S" being the typical and the "R" the mutant form.

8599 P

Enzymatic Digestion of Desiccated Thyroid.

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In the course of studies on the calorigenic activity of various fractions derived from the enzymatic digestion of desiccated thyroid,[1] we have observed that the organic iodine in the gland is rapidly split into acid soluble and acid insoluble iodine fractions. The 2 fractions are separated by adjusting the pH to 5.0.

A single large lot of desiccated thyroid was used for these experiments (Lot No. 2*). The desiccated thyroid was insoluble at pH 5 and contained 2% of the total iodine in the form of "preformed inorganic" iodine as determined by the method of Lawson.[2] When like volumes of enzyme digest were precipitated in dilutions of one, 2 and 4 volumes, the ratio of acid soluble iodine to total iodine was not increased, indicating that the acid insoluble iodine fraction has a negligible solubility at pH 5. The iodine soluble at pH 5 consisted of organic iodine digested off in the acid soluble form and a small amount of "preformed inorganic" iodine. It was thus possible to measure the rate of hydrolysis of desiccated thyroid into acid soluble and acid insoluble iodine fractions, by measuring the ratio of acid soluble to total iodine in the digest.

Incubation of the desiccated thyroid in one and 2% pepsin at pH 2 resulted in a liberation of 55% of the total iodine in an acid soluble form in 4 hours. (The acid soluble fraction is not increased after several months incubation in pepsin solutions.) In one and 2% trypsin at pH 8, 60% of the total iodine was rendered acid soluble in 4 hours. With each enzyme, about 70% of the total hydrolysis into acid soluble and acid insoluble iodine fractions occurred during the first 15 minutes of digestion. Incubation of the desiccated thyroid in human gastric and duodenal juice resulted in the liberation of the acid soluble iodine at the same rate as when concentrated solutions of potent enzymes were used.

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[1] Thompson, W. O., Thompson, P. K., Taylor, S. G., Nadler, S. B., and Dickie, L. F., *J. A. M. A.*, 1935, **104**, 972.

* We are indebted to Dr. D. Klein of the Wilson Laboratories, Chicago, for the desiccated thyroid and enzyme preparations used in these experiments.

[2] Lawson, W., *Biochem. J.*, 1933, **27**, 112.

Since the liberation of amino and carboxyl groups in such digests continues for a number of days,[3, 4] it would appear that one of the first steps in the digestion of desiccated thyroid is the severing of linkages binding the 2 iodine fractions.

The very rapid hydrolysis of desiccated thyroid in peptic and tryptic solutions and in human digestive juices would seem to indicate that therapeutically administered desiccated thyroid is rapidly hydrolysed into acid soluble and acid insoluble iodine fractions.

These data do not seem to support the suggestion of Lerman and Salter[5] that the whole thyroglobulin molecule "is probably absorbed" from the gastrointestinal tract "for the most part unchanged."

8600 C

Influence of Glycine on Depleted Creatine Reserves of Skeletal and Cardiac Muscle in Experimental Hyperthyroidism.*

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The use of glycine in the treatment of muscle disease is based partly on the assumption that it is a precursor of creatine, a number of investigators sharing the view that the transformation of glycine into creatine is direct. In support of the contention that creatine is a product of exogenous protein, or amino acid metabolism, Beard and Boggess[1] have recently presented data to show that the creatine content of muscle of rats may be increased 20 to 56% from the low levels caused by restricted protein intake by refeeding these animals on 25% casein, or egg albumin, or 21%

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[3] Harington, C. R., and Salter, W. T., *Biochem. J.*, 1930, **24**, 456.

[4] Barnes, B. O., Carlson, A. J., and Riskin, A. M., *Am. J. Physiol.*, 1931, **98**, 86.

[5] Lerman, J., and Salter, W. T., *Endocrinology*, 1934, **18**, 317.

* Aided by a grant from the National Research Council.

[1] Beard, H. H., and Boggess, T. S., *Am. J. Physiol.*, 1935, **113**, 647.

glycine, or glutamic acid for a period of 4 weeks. This procedure, which presumably first restricts the intake of creatine precursors and subsequently provides a liberal supply of the same was adopted in pursuit of Rose's suggestion[2] that the origin of an anabolic substance may perhaps be better studied by limiting the intake of a suspected precursor below the required level, rather than by adding excessive quantities to a diet already carrying sufficient amounts to meet the needs of synthesis.

The logic of this approach to the problem is evident although it is to be observed that an increase in the concentration of creatine in muscle and other tissues occurs after feeding creatine.[3] According to Beard and Barnes[4] the same effect may be obtained in normal rats by feeding various amino acids and protein. MacKay[5] found that feeding glycine and *dl*-alanine definitely raised the creatine content of the myocardium, while others,[6] in rabbit-heart perfusion experiments, observed that the addition of glycine to the perfusion fluid produced little, if any, effect, but that alanine caused a definite increase in heart creatine.

In agreement with the work of Brand[7] and others in man and similar studies in experimental animals, one of us has shown[8] that the administration of glycine increases the excretion of creatine in rats. The possibility that this may have no relation to actual production of creatine was emphasized and is further supported by the observation that with the exception of the kidney the creatine content of the tissues is not increased (unpublished data), but on the contrary there is a tendency to loss of creatine from muscle, this occurring in experiments of both short and long duration.

As shown previously[9, 10] hyperthyroidism is associated with a moderate reduction of muscle creatine and a much greater loss from the myocardium. Because of the depleted reserves it was supposed that the tendency to form creatine from its precursors and its retention would perhaps be more readily demonstrable than normally. The present report is based on a study of 68 hyperthyroid rats, the data in the accompanying table representing a number of typical experiments.

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[2] Rose, W. C., *Ann. Rev. Biochem.*, 1933, **2**, 187.

[3] Chanutin, A., and Silvette, H., *J. Biol. Chem.*, 1928, **80**, 589.

[4] Beard, H. H., and Barnes, B. O., *J. Biol. Chem.*, 1931-32, **94**, 49.

[5] MacKay, E. M., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1562.

[6] Decherd, G., Herrmann, G., and Davis, O., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1302.

[7] Brand, E., Harris, M. M., Sandberg, M., and Ringer, I., *Am. J. Physiol.*, 1929, **90**, 296.

[8] Bodansky, M., *J. Biol. Chem.*, 1936, **112**, 615.

[9] Bodansky, M., *J. Biol. Chem.*, 1935, **109**, 615.

[10] Bodansky, M., Pilcher, J. F., and Duff, V. B., *J. Exp. Med.*, 1936, **63**, 523.

Rats 271, 278 and 282 received daily doses of 1 mg. of thyroxine (Hoffman-LaRoche). Muscle was removed for analysis as indicated. Detailed analyses of the urine revealed that following the operation the daily excretion of creatine and creatinine continued at approximately the same level, or slightly above that before the operation. Obviously the continued loss of creatine in these and similar experiments was uncompensated, as the creatine content of the muscle obtained at the conclusion of the experiment was almost invariably lower than the creatine content of the biopsy tissue. The one exception occurred in the case of Rat 282, which died one day after the operation as a result of the exertion incidental to the passage of a stomach tube. The tendency to heart failure in hyperthyroid rats has been commented upon elsewhere.[9, 10, 11] In all 3 animals the creatine concentration of the myocardium had fallen to approximately 50% of normal.

Rats 273 279, 281, 286, 326, and 407 received thyroxine throughout the experimental period. Glycine, one gm. daily, was given by stomach tube following the biopsy as indicated. The addition of amino acids and other substances to the rations was abandoned, because in our experience many rats are sensitive to such changes and consequently eat less food than usual. Analyses of the urine showed an inconstant effect, the excretion of creatine exhibiting wide daily fluctuations, being often depressed sufficiently to suggest a tissue-sparing action. In the aggregate, however, the post-operative excretion of creatine and creatinine exceeded the preoperative excretion. As the concentration of muscle creatine continued to diminish it may be surmized that if any were formed from the administered glycine, it must have been completely destroyed.

Work in this laboratory suggests that the maintenance of the creatinine reserve of muscle, especially of the myocardium, is intimately dependent on the maintenance of an adequate glycogen reserve. The daily administration of one gm. of glucose to the hyperthyroid rats (323, 324, 405) seemed to exert a sparing effect on the tissues. There was a definite lowering of the creatine output, despite the continued administration of thyroxine. However, there was no demonstrable improvement of the creatine content of the

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[11] Bodansky, M., and Pilcher, J. F., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 597.

muscle, but considering the longer survival of these animals, the rate of depletion was perhaps less rapid.

In the rats receiving creatine (275, 283), the tendency to further depletion seemed to be checked somewhat, an effect that occurred more consistently when glucose was given in addition (Rat 329). Only about 40% of the administered creatine was recovered in the urine. As the remainder did not accumulate in the tissues, it may be concluded that a considerable part followed other paths in metabolism.[12] It should be noted that the analyses outlined in Table I were of animals that died, (+) or were sacrificed (S) 18 to 24 hours after the last administration of creatine, glycine, etc.

Guanidine acetic acid produced an effect comparable to that of creatine (Rats 285, 403). This becomes more evident if allowance is made for the considerable proportion of the acid that is excreted unchanged. The extra daily output of creatine due to the guanidine acetic acid was 7-9 mg. in Rat 285 and 4-5 mg. in Rat 403.

When the administration of thyroxine was discontinued, the creatine concentration of the myocardium was rapidly restored, while in the skeletal muscle little change was noted (Rat 327). When creatine was administered during the convalescent period, the concentration in the muscle increased, but restitution in the myocardium was not especially accelerated, comparable results being obtained in the controls, or when glucose was given. As regards the tissue changes convalescence was not strikingly different in the rats receiving glycine than in their controls. In the case of Rat 322, which showed a sharp increase in heart creatine, but no change in the skeletal muscle, the excretion of creatine and creatinine remained practically unaltered during the first 6 days of convalescence, then diminished somewhat. This is in contrast to the reduced creatine output which occurred almost immediately in the control rats and especially in those receiving glucose.

Conclusions. The results of these experiments offer no evidence of the conversion of glycine into creatine. That this amino acid is not a precursor of creatine is indicated by some of the data, but admittedly more conclusive evidence is needed to establish this view. Difficulty in the interpretation of the results is due partly to the fact that the fate of creatine is not limited to storage, excretion, and conversion into creatinine. A large part is used up

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[12] Compare with Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1923, **56**, 29; and Rose, W. C., Ellis, R. H., and Helming, O., *J. Biol. Chem.*, 1928, **77**, 171.

TABLE I.

Rat No. and Sex	Creatine in muscle, mg. per 100 gm.		Myocardium							
	Biopsy specimen	Post-mortem specimen	Wt. of ventricles, gm.	Creatine in ventricles, mg. per 100 gm.	Creatine in ventricles, mg.	Creatine, mg. per 100 gm. Liver	Creatine, mg. per 100 gm. Testes	Thyroxine	Biopsy	Remarks
271 ♂	413	384	1.127	88	.99	24	313	1/9-1/28(+)	1/26	
278 ♀	475	398	.995	101	1.01	42		1/9-2/2(+)	1/26	
282 ♀	414	430	.826	111	.92	39		1/9-1/30(+)	1/29	
273 ♂	328	278	.929	94	.87	29	353	1/9-1/30(+)	1/26	Glycine, 3 daily doses of 1 gm.
279 ♀	420	383	.923	113	1.05	42		1/9-2/7(+)	1/29	” 6 ” ” ” ”
281 ♀	423	356	.918	93	.85	29		1/9-2/2(+)	1/30	” 2 ” ” ” ”
286 ♀	432	366	.965	94	.91	37		1/9-2/9(+)	2/7	” 2 ” ” ” ”
326 ♀	484	435	.980	105	1.03	18		2/8-3/22(S)	3/13	” 7 ” ” ” ”
407 ♂	468	388	1.081	99	1.07	38	398	3/15-3/31(+)	3/28	” 3 ” ” ” ”
323 ♀	458	370	1.103	89	.98	31		2/8-3/24(+)	3/10	Glucose, 13 ” ” ” ”
324 ♀	475	379	.97	107	1.04	22		2/8-3/23(+)	3/11	” 12 ” ” ” ”
405 ♂	473	414	1.02	108	1.10	25	355	3/15-4/5(+)	3/28	” 8 ” ” ” ”
275 ♂	399	382	1.11	81	.90	25	341	1/9-2/29(+)	1/26	Creatine hydrate, 1 daily dose 100 mg.
283 ♀	464	440	.83	130	1.08	29		1/9-2/3(+)	1/29	” ” 3 ” doses ” ”
329 ♀	467	400	1.06	124	1.31	17		2/8-3/26(S)	3/14	” ” 10 ” ” ” ” and glucose, 10 daily doses 1 gm.
285 ♀	450	438	1.07	113	1.21	21		1/9-2/12(+)	1/30	Guanidine acetic acid 10 daily doses 100 mg.
403 ♂	472	445	1.04	105	1.09	39	367	3/15-4/2(+)	3/28	Guanidine acetic acid 4 ” ” 100 ”
327 ♀	435	446	.88	158	1.39	17		2/8-2/26(S 3/6)	2/26	
321 ♀	451	486	.96	143	1.37	18		2/8-3/4(S 3/14)	3/5	Glucose, 8 daily doses 1 gm.
322 ♀	434	436	.92	178	1.64	18		2/8-2/27(S 3/6)	2/27	Glycine, 8 ” ” ” ”
292 ♀	425	488	.99	144	1.43	19		1/30-3/4(S 3/15)	3/5	Creatine hydrate, 7 daily doses 100 mg.

in other ways. Moreover, in hyperthyroidism, as in certain muscle diseases,[13] the capacity of muscle to take up and retain creatine is deficient, even though the concentration may be considerably below normal.

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[13] Bodansky, M., Schwab, E. H., and Brindley, P., *J. Biol. Chem.*, 1929, **85**, 307.

Peiping Section

Peiping Union Medical College, February 19, 1936.

8601 C

Response to Neoarsphenamine of Wassermann Antibody Induced in Rabbits by Injection of Normal Hamster Tissues.

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A recent study of a case of paroxysmal hemoglobinuria[1] has aroused our interest in the question of the etiology of this disease. The presence of Wassermann antibody in the serum of patients with paroxysmal hemoglobinuria and its response to neoarsphenamine have led the majority of workers to the belief that syphilis is the etiological factor. The behavior of a non-syphilitic Wassermann antibody towards injections of neoarsphenamine has not so far been studied.

Wakerlin and Carroll[2] found that rabbits receiving intratesticular injection of *Treponema pallidum* developed a positive Wassermann reaction after an average of 5 to 6 weeks, and that the Wassermann antibody gradually disappeared from the blood so as to give a negative reaction in an average of 84 days. At this time syphilitic infection goes into a latent stage in rabbits. In treating 9 rabbits with neoarsphenamine, they found that the Wassermann reaction became negative in an average of 28 days after treatment, while in the 6 controls receiving no neoarsphenamine the average duration of positive reaction was 94 days. The duration of positive Wassermann reaction in treated syphilitic rabbits was therefore shortened to one-third of that in untreated controls.

Recently, Hu and his co-workers[3] found that normal rabbits receiving injections of hamster tissues developed strongly positive Wassermann reactions. This Wassermann antibody was definitely non-syphilitic in nature as the injection consisted only of normal hamster tissues in which syphilitic virus could be excluded. The response of this antibody to neoarsphenamine was not studied at the time. The present report deals with the results of such a study.

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[1] Huang, C. H., and Sia, R. H. P., *Chinese Med. J.* (To be published).

[2] Wakerlin, G. E., and Carroll, P. H., *Arch. Derm. and Syph.*, 1925, **12**, 670.

[3] Hu, C. K., Wong, D. H., and Pearce, L., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 989.

Eleven male rabbits were used in the present experiment. All rabbits received one or 2 cc. of a tissue-emulsion of normal hamster's testis or brain either intratesticularly or intraäbdominally. Wassermann and Kahn tests were performed weekly on the blood of all rabbits before and after the injection. The Wassermann reactions in all rabbits which were found to be negative before the injection became positive after an average of 3 weeks (the shortest being 2 weeks and longest 7 weeks), and the Kahn reactions became positive after an average of 2 weeks (the shortest being one week and longest 5 weeks). Three weeks after the injection of hamster tissues, 6 rabbits received neoarsphenamine (0.015 gm. per kg. of body weight) in distilled water intravenously, and 5 rabbits, serving as controls, received at the same time equivalent amounts of distilled water only. The injections of neoarsphenamine were repeated at weekly intervals and the Wassermann and Kahn tests were applied to specimens of blood obtained before each treatment.

Results. Wassermann reaction: In the control series, the duration of positive Wassermann reactions was found to vary between 14 and 38 days, with an average of 31.2 days in the 5 rabbits tested. In the group receiving neoarsphenamine, the duration of positive reactions varied between 7 and 38 days, with an average of 30.2 days.

Kahn reaction: This remained positive for 28 to 45 days in the control group, with an average of 37 days. In the group receiving neoarsphenamine, positive Kahn reactions persisted for 28 to 45 days also, with an average of 37.2 days. Two rabbits from each group still showed positive Kahn reactions 45 days after the injection of neoarsphenamine or distilled water.

The experiment is conclusive in showing that the non-syphilitic antibody as induced in rabbits by the injection of normal hamster tissues does not respond to injections of neoarsphenamine. The average duration of positive Wassermann and Kahn reactions was practically the same in both groups of animals. This is quite different from the behavior of the syphilitic Wassermann antibody as induced in rabbits by the injection of *Treponema pallidum*. The duration of positive Wassermann reactions in the latter case can be considerably shortened by treatment of the animals with neoarsphenamine.

Summary. Rabbits receiving normal hamster tissues intratesticularly or intraäbdominally developed, in the course of about 3 weeks after the injection, Wassermann and Kahn antibodies. These non-syphilitic antibodies persisted in the blood stream of these animals for about 4 to 5 weeks.

Injections of neoarsphenamine given to these rabbits did not shorten or modify the duration of positive Wassermann and Kahn reactions in these animals.

8602 C

Experimental Virus Infections in Chinese Hamster. I. Susceptibility to Fixed Rabies Virus.

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The characteristics of most viruses are best demonstrable by their effects on hosts. It is therefore desirable to determine the pathogenicity of a given virus for as many species of animals as possible. It seems to us of both practical and academic interest to find out the effect of various viruses upon the Chinese hamster, a species of rodent readily obtainable. In the present communication, the susceptibility of the Chinese hamster to fixed rabies virus is recorded.

A strain of fixed rabies virus after 137 rabbit passages from a local street virus, was used. The brain virus preserved in 50% glycerin was thoroughly ground in a sterile mortar and suspended in saline solution. Normal Chinese hamsters weighing 20-30 gm. were divided into several groups and each group was separately inoculated with various dilutions of the rabbit brain virus through different routes. In intracerebral inoculation, the hamsters were anesthetized and 0.05 cc. of the ground virus suspension was injected through an opening in the skull posterior to the eye and lateral to the mid-dorsal line. In intraperitoneal, intratesticular, intramuscular and subcutaneous injections, 0.5 cc. of virus suspension was introduced each time. The latter 2 routes of injection were always made over the right thigh, Animals, after injection, were kept separately in cages and observed for over one month.

After intracerebral inoculations, the infected animals began to show general weakness and tremor of different parts of the body in 5 to 8 days. This was followed by progressive unsteadiness of gait accompanied by weakness of the hind-legs, leading in 24 hours to definite paralysis. The upper limbs were found to be involved later. At the terminal stage all limbs became paralyzed, respiratory movements feeble and the skeletal muscles flabby. Definite convulsions usually appeared spontaneously, but occurred more frequently when the animals were irritated. When the infective dose was moderate (0.05 cc. 0.1% virus suspension) the animal died in 2-3 days after appearance of weakness of the hind-legs. When the infective dose was large (0.05 cc. 10% virus suspension) animals often died within 24 hours after onset of the general weakness, and no definite paralytic symptoms could be observed. In a few animals, symptoms of excitement were especially noticeable. They manifested the first sign of disease by jumping about in the cage almost continuously until exhausted. They did not show any special desire to bite, but, when irritated, would bite another animal introduced into the same cage. A few normal animals so bitten were observed for one month without showing any symptoms. The stage of excitement usually lasted for one or 2 days, being followed by profound general weakness and death within a few hours.

After other routes of inoculation, the infected animals showed symptoms and incubation periods similar to those shown by a moderate dose of the virus intracerebrally. All of these animals showed paralysis of hind-legs and rarely died within a day after paralysis was seen. None showed marked spontaneous excitability. Regardless of the routes of inoculation animals invariably died within 3 days after the onset of paralysis. Animals showing no symptoms and surviving over a month were considered to have escaped infection.

The percentages of death and periods of survival of infected animals are given in the accompanying table. The intracerebral route was found to be the most highly effective as some hamsters could still be infected with the original virus suspension diluted to 2 million parts. The other 4 routes of inoculation were far less effective as the infection was possible only with much more concentrated suspension of the virus. Of these 4, the intramuscular

TABLE I.
Showing Comparative Infectivity of Fixed Rabies Virus Introduced Through Different Routes.

Routes of inoculation	Dilution of brain virus	No. animals inoculated	No. animals died	% death	Day of deaths	Aver. day of deaths
Intracerebral	$1:2 \times 10^4$	19	19	100	5, 5, 6, 6, 6, 6, 7, 7, 7, 7, 7, 7, 8, 8, 9, 9, 9, 9, 10	7.3
	$1:1 \times 10^5$	12	12	100	7, 7, 8, 8, 8, 8, 9, 9, 9, 10, 10, 10	8.6
	$1:5 \times 10^5$	10	8	80	7, 8, 8, 8, 8, 8, 10, 10	8.4
	$1:1 \times 10^6$	10	5	50	8, 8, 9, 10, 10	9.0
	$1:2 \times 10^6$	10	2	20	10, 11	10.5
Intraperitoneal	1:10	10	3	30	7, 7, 8	7.3
	$1:1 \times 10^2$	9	1	11	10	10.
	$1:1 \times 10^3$	11	0	0	—	—
Subcutaneous	1:10	10	5	50	8, 9, 10, 10, 10	9.4
	$1:1 \times 10^2$	10	1	10	10	10.
	$1:1 \times 10^3$	12	0	0	—	—
Intramuscular	1:10	10	8	80	7, 8, 8, 9, 10, 10, 10, 9	8.9
	$1:1 \times 10^2$	10	6	60	7, 8, 8, 10, 10, 11	9.0
	$1:1 \times 10^3$	10	4	40	8, 9, 10, 10	9.3
Intratesticular	1:50	8	4	50	7, 8, 9, 9	8.3
	$1:1 \times 10^2$	7	0	0	—	—
	$1:1 \times 10^3$	7	0	0	—	—

route was slightly more effective than the other 3, and the intratesticular route was the least effective. It is of interest to note that no marked variation in the period of survival in relation to different routes of injection was observed, as one would expect from general experience in larger animals; on the other hand, its relation to the dose of inoculation was suggested. For instance, the average day of death was 7.3 days for the group injected intracerebrally with 1:20,000 dilution of the original virus suspension, and was increased to 9.0 days when the virus suspension injected was diluted to a million. However, when comparable concentration of virus inoculation was taken into consideration, the results suggested a slight lengthening of the time until death when the virus was given through routes other than the intracerebral one.

While most workers[1] have not been able to demonstrate Negri bodies in animals infected with fixed rabies virus, others[2] have claimed occasional success in certain species of animals. In view of this, it seemed of interest to ascertain whether Negri bodies could be found in hamsters infected with fixed rabies virus. Brains from hamsters in the paralytic stage of the disease 6 days after inoculation with the fixed virus either intracerebrally or subcutaneously were fixed for 24 hours in 95% alcohol containing 5% acetic acid. The sections stained by Harris' hematoxylin and eosin showed in the ganglion cells of Ammon's horn the presence of Negri bodies which were especially numerous in the Purkinje cells. The Negri bodies averaged 3 x 4 μ in size, but occasionally some as large as 4 x 6 μ were found.

From the brain of infected hamsters, the virus could be recovered. Up to the present, the virus has been carried to the 10th passage, and it has shown no significant change in pathogenicity.

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[1] Levaditi, C., Nicolau, S., et Schoen, R., *Ann. Inst. Pasteur*, 1926, **40**, 973.

[2] Palawandow, H., Serebrennya, A. I., and Pugatsch, E. M., *Z. f. Hyg. u. Infektionskr.*, 1934, **116**, 433.

8603 P

A New Preparation Protecting Against Rat Liver Necrosis.

J. C. Forbes and R. C. Neale. (Introduced by H. B. Haag.)

From the Department of Biochemistry, Medical College of Virginia, Richmond.

A preparation has been obtained from hog liver which exerts a marked protective action against liver necrosis produced in rats by carbon tetrachloride inhalation. Also preliminary experiments indicate that the substance definitely lessens the cirrhotic changes in the liver resulting from chronic carbon tetrachloride poisoning.

Preparation of material: To a concentrated aqueous extract of hog liver (about 29° Baumé), sufficient alcohol was added to make an 80% alcoholic solution and the solution filtered. The material precipitated from the filtrate by an excess of silver nitrate was treated with concentrated hydrochloric acid and the precipitated silver chloride removed. The supernatant fluid was neutralized until alkaline to congo red but acid to litmus, and evaporated under reduced pressure to a small volume. Inorganic salts were removed by the addition of alcohol to 80%, and filtration. The alcohol was evaporated off under reduced pressure and the residual liquid diluted with distilled water so that 1 cc. was equivalent to approximately 60 gm. of fresh liver.

The subcutaneous injection of this material into rats not only greatly reduced the degree of necrosis and fatty degeneration produced in acute poisoning, but also appears to speed up the regenerative processes. The mechanism of the protective action is not known but experiments show that the beneficial effect cannot be ascribed to the small amount of glucose present in the relatively impure preparation.

In the experiments with acute carbon tetrachloride poisoning, rats weighing approximately 125 gm. each were given daily subcutaneous injections of one cc. of the protective material, beginning 5 or 6 days prior to the day of acute poisoning. An equal number of rats were used as controls. Treated and control rats were subjected to inhalation of carbon tetrachloride. The rats were placed in a cage at the bottom of a large box, relatively air tight, with glass top and side. Carbon tetrachloride-laden air was forced into the box through a small opening near the top until the rats were semiconscious. They were kept in this state for periods varying from 2 to 2½ hours.

About 18 hours after the administration of the carbon tetrachloride, a control and a treated rat were killed. Thereafter, at least one control and one treated animal were killed each day over a period of 5 or 6 days. Immediately after the animals were killed, a portion of each liver was placed in formalin solution and later sectioned for microscopic studies.

Sections from the livers of the control rats showed extreme fatty degeneration and necrosis while the livers of the protected animals, with very few exceptions, were only slightly damaged. Even in these rats, the amount of degeneration did not approach that of the least damaged corresponding controls. Over 50 pairs of rats have been used in these acute poisoning experiments.

8604 C

Non-Effect of Estrogenic Hormones on Mammary Gland of Hypophysectomized Guinea Pig.*

E. T. GOMEZ AND C. W. TURNER.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.†

It was reported[1] that the mammary gland duct system of immature hypophysectomized albino rats failed to develop when dosages of 25 to 500 I. U. per day of the estrogenic hormone (progynon-B) were injected. To determine whether our results represented a species difference or applied generally, the study has been extended to other species, including the guinea pig, mouse, rabbit and cat. The present paper will present the observations with guinea pigs.

Since our work was begun two conflicting reports upon the subject have appeared. Nelson[2] reported mammary development in four male hypophysectomized guinea pigs, treated with 40 R. U. of oestrone daily, comparable to that observed in normal guinea pigs. It is not clear from his report whether he refers to the growth of an extended duct system or to a lobule-alveolar system, but as estrogenic hormone normally stimulates the growth of the lat-

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* Aided in part by a grant of the Committee on Grants-in-Aid of the National Research Council.

† Journal Series No. 459.

[1] Reece, R. P., Turner, C. W., and Hill, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 204.

[2] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 222.

TABLE I.

Non-effect of the Estrogenic Hormone on the Mammary Glands of Hypophysectomized Male and Female Guinea Pigs.

Animal No.	Sex	No. Days of Injections	Estrogenic Hormone.		Observations	
			Kind	Daily Dose I.U.	Nipples	Gland
G35-12	F	40	Theelin (aqueous)	100	Well developed	No duct growth
G35-1	F	32	Progynon-B	100	" "	" " "
G35-3	F	65	" "	150	" "	" " "
G35-4	M	25	" "	1000	" "	" " "
G36-12	F	20	Theelin in oil	50	" "	" " "
364	M	30	" " "	50	" "	" " "
362	F	30	" " "	50	" "	" " "
363	M	25	" " "	25	" "	" " "
365	M	20	Oestroform-B	50	" "	" " "
			Incompletely Hypophysectomized			
G35-14	M	25	Progynon-B	100	" "	Complete hyperplasia of lobule-alveolar system
G35-15	M	30	" "	100	" "	Complete hyperplasia of lobule-alveolar system
G35-16	F	35	Theelin (aqueous)	100	" "	Complete hyperplasia of lobule-alveolar system
G35-13	M	30	"	100	" "	Complete hyperplasia of lobule-alveolar system

ter in intact guinea pigs[3] it is assumed that complete development was observed. On the other hand, Lyons and Pencharz[4] observed nipple development in 4 hypophysectomized male guinea pigs after 2 months of progynon-B injections almost equal to that which they obtained in 6 normal male animals under similar treatment, yet there was only a limited growth of ducts in the hypophysectomized animals in comparison to functional alveolar development found in the injected intact males.

A total of 9 young male and female guinea pigs have been injected immediately after hypophysectomy with varying amounts of the estrogenic hormones, theelin,‡ both aqueous and in oil and the benzoate of dihydrotheelin (estroform-B and progynon-B) for periods varying from 20 to 65 days. While these injections stimulated extensive development of the nipples even in the male, the mammary duct system failed to grow (Table I). In 4 additional animals, which upon postmortem examination were found to be incompletely hypophysectomized, similar treatment showed the growth of an extensive lobule-alveolar system. These observations confirm and extend the findings of Lyons and Pencharz.

Conclusion: While the growth of the nipples of the hypophysectomized guinea pig may be stimulated with the estrogenic hormones, theelin and the benzoate of dihydrotheelin, neither the ducts nor the lobule-alveolar complex of the mammary gland responded even to long continued administration. It would thus appear that the inability of the estrogenic hormone to stimulate the growth of the mammary gland parenchyma in the absence of the hypophysis is probably not a species difference.

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[3] Turner, C. W., and Gomez, E. T., *Mo. Agr. Exp. Sta. Res. Bul.*, 206, 1934.

[4] Lyons, W. R., and Pencharz, R. I., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 589.

‡ Theelin was kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co., and progynon-B by Dr. E. Schwenk of the Schering Corporation.

8605 P

Chemical Treatment of Tumors. III. Separation of Hemorrhage-Producing Fraction of *B. coli* Filtrate.

M. J. SHEAR AND H. B. ANDERVONT.

From the Office of Cancer Investigations, U. S. Public Health Service, Harvard Medical School, Boston.

Several investigators have found that hemorrhage could be produced in tumors by the administration of bacterial preparations and that the tumors subsequently receded in a significant proportion of cases. Confirmation of these findings has been reported.[1, 2] However, the results obtained were not satisfactory inasmuch as small doses were often ineffective and large doses were toxic. The separation of the hemorrhage-producing agent from toxic and inert contaminants was therefore undertaken.

Little progress has hitherto been reported in this direction. The most recent relevant contribution is that of Apitz[3] who attempted to purify the "Shwartzman-active" substances present in *B. typhosus* and *B. paratyphosus* B preparations. Partial purification was effected, but Apitz found that the Shwartzman-active material so obtained was highly unstable; this instability halted further purification. It should be pointed out however that, in this study, Apitz was concerned solely with the Shwartzman phenomenon and reported no observations on the effect of this material on tumors.

The agent in *B. coli* filtrate responsible for hemorrhage production in mouse tumors was found to be stable in most instances, in this laboratory, even after a high degree of purity had been attained. The first steps in the separation of the active fraction were based upon the method devised by Felton, Kauffmann and Stahl[4] for the precipitation of the soluble specific polysaccharide from pneumococcus broth cultures.

The method used was, briefly, as follows:

The organisms were removed from week-old nutrient broth by centrifugation and by Berkefeld filtration. Phosphate was added to the filtrate; then the pH was brought to 9.5 by addition of lime water. The active material was carried down with the basic cal-

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[1] Shear, M. J., and Andervont, H. B., Ann. Rep. of Surgeon General, Public Health Service of U. S., Washington, D. C., p. 25, 1932.

[2] Shear, M. J., *Am. J. Cancer*, 1935, **25**, 66. (This paper also contains a review of the literature.)

[3] Apitz, K., *J. Immunology*, 1935, **29**, 343.

[4] Felton, L. D., Kauffmann, G. and Stahl, H. J., *J. Bact.*, 1935, **29**, 149.

cium phosphate precipitate, but a single precipitation did not always suffice to bring it down completely. In those cases in which precipitation failed to remove all activity from the supernatant, more phosphate was added and the pH again brought to 9.5 with lime water. This was repeated until all of the active material was removed from solution.

The calcium phosphate precipitates were combined, and dissolved in hydrochloric acid. Insoluble material was filtered off and discarded. Reprecipitation was then effected by the addition of sodium hydroxide to pH 9.5. The supernatant solution was inactive, as a rule, and was usually discarded. The precipitate was again dissolved in acid and again precipitated with sodium hydroxide. The object of these reprecipitations was to remove whatever protein had been carried down in the original precipitation.

The final calcium phosphate precipitate was then treated with acid and alcohol to remove the calcium. Enough hydrochloric acid was added to dissolve the precipitate and to bring the solution to pH 2. Insoluble matter was filtered off and discarded. The active material was precipitated from solution by addition of ethyl alcohol; the calcium remained in solution. Solution in acid and precipitation with alcohol was repeated until the precipitate was free from calcium. At times, anhydrous sodium acetate was added to the acid alcohol to promote complete precipitation of the active material.

This fraction was dissolved in water, neutralized with sodium hydroxide and evaporated to dryness. Finally, excess sodium chloride was removed by repeated treatment with methyl alcohol.

The purification was guided by testing the activity of each fraction on tumor-bearing mice. Since Andervont[5] had found that different strains of mice reacted somewhat differently to this agent, regularly reproducible results were insured by the employment of pure strain mice; most of the testing was done with Strain M mice. The fractionation was facilitated by the use of Andervont's skin-tumor technique[5] which provided a rapid, quantitative method of assaying activity. This made it possible to complete a determination of activity within 5 hours. For the most part, sarcoma 37 was employed as the test tumor although other transplanted sarcomas, as well as primary sarcomas induced by polycyclic hydrocarbons, were similarly affected by the agent.

By this procedure a water soluble fraction was obtained from *B. coli* filtrates which was highly potent in producing hemorrhage in mouse tumors. The minimum effective dose was 0.1 cc. of a

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[5] **Andervont, H. B., *Amer. J. Cancer* (In Press).**

1:1,000 dilution of a stock solution which contained 22 mg. of the active fraction per cc. Hemorrhage was produced regularly by this dose, which contained 0.0022 mg. of active material.

8606 P

Chemical Treatment of Tumors. IV. Properties of Hemorrhage-Producing Fraction of *B. coli* Filtrate.

M. J. Shear.

From the Office of Cancer Investigations, U. S. Public Health Service, Harvard Medical School, Boston.

The active fraction obtained from *B. coli* filtrate by the method described in the preceding paper was dissolved in water and evaporated to dryness *in vacuo*. A white residue was obtained, largely crystalline in nature. Strong heating produced some charring, but much of the crystalline residue was unaffected indicating that a considerable portion was inorganic in nature.

On repeated treatment of the active fraction with methyl alcohol, most of it (82%) went into solution. The methyl alcohol solution was evaporated to dryness and taken up in water, but most of it now failed to dissolve. However, addition of sodium hydroxide effected solution. Acidification with hydrochloric acid produced a heavy, flocculent precipitate which dissolved again on further addition of alkali. This solution was neutralized and tested on tumor-bearing mice; it was completely inactive as far as production of hemorrhage was concerned.

The methyl alcohol-insoluble portion (18%) dissolved readily in a small volume of water, with formation of a permanent foam. The presence of surface tension reducing material had been noted early in the purification, and its occurrence paralleled the presence of the active agent in the course of the fractionation. A stock solution was prepared containing 1 mg. per cc. of this fraction. This solution produced hemorrhage in mouse tumors in doses of 0.2 cc. of a 1:500 dilution, *i. e.,* the minimum hemorrhage-producing dose contained 0.0004 mg. of active material.

Upon evaporation to dryness *in vacuo* the active solution gave a residue which was largely non-crystalline. A comparatively small amount of crystalline material was present in which 3 crystal types were noted; the bulk of the residue consisted, however, of a film of non-crystalline glassy material.

The above-described stock solution of the active fraction gave a negative biuret test and a strongly positive Molisch test. It is interesting to note that the partially purified Shwartzman-active substances obtained by Apitz[1] also gave a negative biuret and a positive Molisch test.

The absorption spectrum of the active fraction was examined by Dr. E. Lorenz; no selective absorption was noted.

Since the most striking immediate effect of this agent on tumors is the production of severe hemorrhage, the reaction of the vascular system to this agent is of interest. Dr. V. Menkin[2] of the Pathology Department of the Harvard Medical School tested its effect on the permeability of capillaries of normal tissue; no increase in permeability was detected.

The purified fraction, in both concentrated and dilute solution, retained its activity after storage for 8 weeks in the cold room.

8607 P

Differential Bile Acid Analysis in Various Pathological Conditions.

HENRY DOUBILET AND RALPH COLP. (Introduced by Louis Gross.)

From the Laboratories, Mount Sinai Hospital, New York City.

By means of a method previously described,[1] bile from normal and diseased gall bladders and from biliary tract fistulae was analyzed for bile acids conjugated with taurine and with glycine, cholic acid, deoxycholic acid, total bile acids, and free bile acids. Duodenal drainage material was analyzed for cholic acid, deoxycholic acid and total bile acids. Some of the results are summarized in Table I.

A perusal of Table I shows that in a series of 24 cases of chronic cholecystitis (No. 2, Table), the average figure for the percentage of cholic acid in relation to the total bile acids was 28% as compared to the normal of about 50%. The average figure for the ratio of free bile acids rose from a normal of about 10% to 25%. This deviation in the ratio of the different bile acids is more marked in the gall bladder bile in acute cholecystitis. In 14 of these cases (No. 3, Table) the cholic acid averaged only 13% of the total bile acids. It was found difficult to estimate the free bile acids in a number of cases, since gall bladder bile from acute cholecystitis cases often contained an alcohol soluble material which yielded amino nitrogen on hydrolysis. This not only gave a false result, but also masked the presence of free bile acids. Two typical cases are presented in the table. In the first, (No. 3A) 75% of the total was found to be free bile acids; in the second case, (No. 3B) estimation of bile acids by the Schmidt-Dart method (nitrogen hydrolysis) yielded a figure much greater than the total bile acids. At the same time no trace of cholic acid was found.

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[1] Apitz, K., *J. Immunology*, 1935, **29**, 343.

[2] Personal communication.

[1] Doubilet, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 86.

These figures show that in chronic cholecystitis the average reduction of the total bile acids is approximately 50% while in acute cholecystitis, the total bile acids are reduced on the average by 90%.

From these figures it appears that the absorption of bile acids by an inflamed gall bladder wall[2, 3] proceeds in a differential manner. The cholic acid disappears more rapidly than the deoxycholic acid, while the conjugated bile acids tend to be absorbed at a faster rate than the free bile acids.

In early biliary obstruction due to carcinoma of the pancreas, the bile salts tend to accumulate and reach very high figures, (No. 4, Table). In these cases, the ratio of cholic acid and of free bile acids to the total tend to be normal, although at times the percentage of free acids is quite high (No. 4A). When the obstruction is of long duration (No. 4B) and absorption obviously has taken place, the total percentage of bile acids tends to be very low. Here again the ratio of cholic acid to the total is low, while there is a high percentage of free bile acids.

In cases of cholangitis, analysis of the fistula bile tends to yield figures which are analogous to those obtained from the gall bladder bile in cases of acute cholecystitis. The percentages of cholic acid and of free bile acids as compared to the total bile acids are similar to those in acute cholecystitis. A typical example is found in the Table (No. 5). In the absence of cholangitis, after recovery from the operative procedure, analysis of fistula bile reveals normal proportions in the different bile acids present.

When tubes drain both the gall bladder and the common bile duct at the same time, analysis of the bile collected simultaneously shows clearly the remarkable ability of the inflamed gall bladder to absorb bile salts (No. 6, Table). The total amount of bile acids

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[2] Rosenthal, F., and Licht, H., *Klin. Wchnschr.*, 1928, **7**, 1952.

[3] Andrews, E., Schoenheimer, R., and Hrdina, L., *Arch. Surg.*, 1932, **25**, 796.

TABLE I.
Differential Quantitative Bile Acid Analysis in Various Conditions.

Group	No. of Cases	Conjug. with Taurine %	Conjug. with Glycine %	Cholic Acid %	Deoxycholic Acid %	Cholic Acid %	Free Bile Acid %
1. Normal	3	3.05	4.06	3.74	4.12	48	10
2. Cholecystitis (C)	26	1.53	1.74	1.19	3.15	28	25
3. Cholecystitis (A)	14	—	—	0.10	0.64	13	—
*A		0.38	0	0.23	1.34	14	75
*B		0.32	0.11	0	0.32	0	—
4. Ca. Pancreas							
A	3	2.83	4.18	4.67	4.97	50	29
B	2	0.10	0.02	0.03	0.10	23	8
5. *Cholangitis		0.11	0	0.03	0.21	12	54
6. *Simultaneous							
A		0.15	0.15	0.16	0.14	53	0
B		0.36	0.01	0.01	0.01	50	—
7. *Simultaneous							
A		0.59	0.77	0.32	0.52	37	—
B		—	—	0.24	0.48	34	—
8. *Simultaneous							
A		—	—	0.83	1.87	31	—
B		—	—	0.10	0.09	52	—
C		—	—	1.37	2.84	33	—
D		—	—	0.10	0.19	35	—
E		—	—	0.02	0.29	6	—
F		—	—	0.13	0.22	37	—

*Typical case.

is reduced 10 times in passing through the gall bladder (No. 6A) as compared to the bile acids collected from the common bile duct (No. 6B). Simultaneous collection of bile from a common duct fistula (No. 7A) and by means of a duodenal drainage (No. 7B) shows the dilution produced by pancreatic juice and succus entericus. Other analyses have shown 2 or 3 fold dilution of the hepatic bile.

In a number of cases duodenal drainage was performed immediately before operation for gall bladder disease, and the gall bladder bile analysis compared with the duodenal bile. As a rule the duodenal "A" bile showed a normal proportion of cholic acid (No. 8B) while the gall bladder bile, in chronic cholecystitis, had a ratio of cholic acid to total bile acids of about 30% (No. 8A). Occasionally analysis of duodenal "A" bile showed an abnormally low ratio of cholic acid (No. 8D) while the bile removed from the gall bladder immediately afterwards showed the typical low cholic acid ratio (No. 8C). In acute cholecystitis the percentage of cholic acid was always much lower in the gall bladder bile (No. 8E) than in the duodenal drainage "A" bile (No. 8F, Table).

8608 P

Effect of Light on Reproductive Cycle of *Peromyscus leucopus noveboracensis.*

WAYNE L. WHITAKER. (Introduced by H. B. Lewis.)
From the Department of Zoology, University of Michigan.

Bissonnette[1] apparently eliminated exercise as a causative factor in advancing the breeding season of his starlings, showing that such an advance was due to the effect of the added light acting on the birds more directly. The same investigator[2, 3] has reported that artificial light added to normal light has a similar effect on the ferret. These mammals showed gametogenesis, active development of reproductive organs, libido, and oestrus several weeks in advance of the controls.

A similar study is under way on the white-footed mouse, *Peromyscus leucopus noveboracensis* (Fischer). Credit is due Dr. Alvalyn E. Woodward for her advice on the conduct of this study.

Animals, trapped locally during the winter of 1934-35, were used, along with their mature offspring. Two groups were kept in a large basement room. Room conditions of natural light, heat, humidity, and ventilation were the same for both groups. The mice were kept in wire cages, usually in pairs. The diet was that of Dice.[4]

Group A, consisting of 35 animals, was given about 13 hours daily illumination from a General Electric Sunlamp Type S-1, 6 feet from nearest cages, starting September 23, 1935. The light was gradually increased up to 18 hours daily by December 26. Group B, the control group, consisting of 28 animals, was treated with an electric radiant heater to compensate for the heating effect of the lamp on Group A.

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[1] Bissonnette, T. H., *J. Exp. Zool.*, 1931, **58**, 281.

[2] Bissonnette, T. H., *Proc. Roy. Soc., B.*, 1932, **110**, 322.

[3] Bissonnette, T. H. *Biol. Bull.*, 1935, **68**, 300.

[4] Dice, L. R., *J. Mammal.*, 1934, **15**, 160.

The criteria for establishing the sexual season were descended or enlarged testes and open vagina. The testes are too small to palpate and the vagina is completely closed during anoestrum. The percentages of individuals of each group in sexual season at the indicated dates are plotted on the accompanying graph.

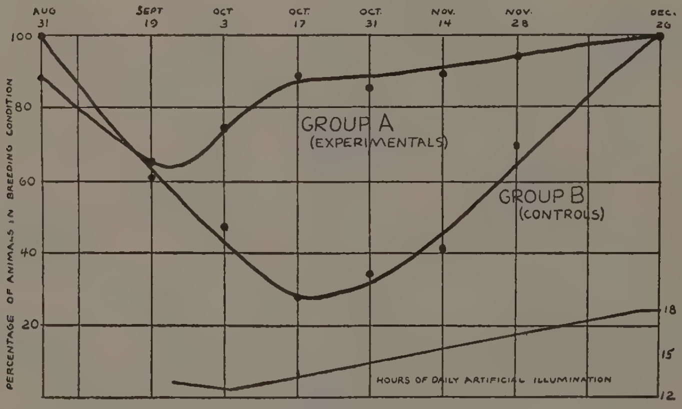

FIG. 1.

Graph showing the percentage of mice of each group in sexual season at the indicated dates, and hours of daily artificial illumination on Group A.

Group A was about 6 to 8 weeks ahead of the control group in advancing into the sexual season. During the course of the experiment, 4 pairs of mice in Group A delivered 5 litters while there were no deliveries in the control group. It would seem from these results that there is some relationship between light and the reproductive cycle of the form studied. Food consumption and body activity apparently increase, while weights decrease slightly as the sexual season comes on. Average body weights of the 2 groups were very nearly equal.

This experiment is being repeated with lower and better controlled temperatures, with distinction between intensity and duration of light, with more frequent examination of the animals, with consideration to food consumption, body weight, and activity of the individuals, and with histological studies.

8609 P

Light Regulation of Sexual Activity in the Male Sparrow (*Passer domesticus*).*

Gardner M. Riley. (Introduced by E. Witschi.)
From the Zoological Laboratory, State University of Iowa.

The importance of light as a regulating factor of the sexual cycle in birds has been demonstrated by Rowan[1] (junco), Bissonnette[2] (European starling), Miyazaki[3] *(Zosterops palpebrosa)* and Kirschbaum[4] (English sparrow). These investigators have observed a precocious development of the testes following daily increases in the period of illumination. Such experiments were usually carried out during the early winter. The present author has undertaken to determine whether lengthened daily periods are equally effective at a time further removed from the normal breeding season. In this paper, a group of male sparrows, exposed to additional light beginning November 18th, is compared with another group first exposed on September 30th.

The November group of sparrows had been in the laboratory 16 days previous to the artificial lengthening of the days and all possessed the horn-brown bill of the sexually quiescent period. The birds received 4½ additional hours of light, daily, from a 100-watt incandescent bulb throughout the experiment, increasing the day to a total of about 14 hours. The effect of such treatment both on bill color and testis is shown in Table I.

The acceleration of testicular development which was observed in the birds of this group is in full agreement with the results of the aforementioned authors.

A second group of male sparrows, however, (Table II) first subjected to a lengthened light period on September 30th, gave a decidedly different result. These birds had been in the laboratory 10 days prior to experimental use. The light period was extended 2 hours the first day, 3 hours the second and from this time on 15 additional minutes daily until, after 14 days, the period had been increased 6½ hours, making a total of approximately 17 hours

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Emil Witschi.

[1] Rowan, W., The Riddle of Migration, Baltimore, 1931.

[2] Bissonnette, T. H., *J. Exp. Zool.*, 1931, **58**, 281.

[3] Miyazaki, H., *Sci. Rep. Tohoku Imp. Univ.*, IV, 1934, **9**, 183.

[4] Kirschbaum, A., *Anat. Rec.*, 1933, **57**, Supp., 62.

TABLE I.
Effect of Increased Light Periods on Sparrows, Starting November 18, 1934.

Bird	Bill color after 17 days	Bill color after 26 days	Bill color after 41 days	Final size of L. testis, mm.	Final condition of testes
		Experimental Group			
Y-1	I	Bird died		1.3 x 1.0	Quiescent spermatogonia
Y-2	I	II	III	7.0 x 6.0	Mature spermatozoa
Y-3	I	II	III	6.9 x 5.0	” ”
Y-4	I	II	III	7.2 x 6.0	” ”
Y-5	I	II	III	6.9 x 5.2	” ”
Y-6	I	I	II	5.0 x 4.0	Few spermatozoa
		Control Group			
YC-1	Killed at start of experiment			1.2 x 1.0	Quiescent spermatogonia
YC-2	” ” ” ” ”			1.5 x 1.1	” ”
YC-3	0	0	0	1.2 x 0.9	” ”
YC-4	0	0	0	1.9 x 1.4	” ”
YC-5	0	0	0	1.3 x 1.1	” ”

0, no change; I, darkened brown; II, light blue; III, dark blue (Witschi[5]).

TABLE II.
Effect of Increased Light Periods on Sparrows, Starting September 30, 1935.

Bird No.	L. testis before exposure, mm.	Days of exposure	L. testis after exposure, mm.	Final bill color	Final condition of testes
80	2.0 x 1.5	18	2.0 x 1.7	0	Quiescent spermatogonia
70	2.2 x 1.2	18	1.5 x 1.2	0	Slight degeneration
77	2.4 x 1.7	39	2.6 x 2.0	0	Quiescent spermatogonia
97	— —	39	1.0 x 0.6	0	” ”
74	1.4 x 1.0	50	1.8 x 1.1	0	” ”
98J	1.0 x 0.7	23	4.6 x 3.2	I	Spermatocytes
78J	1.9 x 1.4	23	7.7 x 5.0	II	Spermatozoa
65J	1.2 x 0.9	39	6.8 x 5.0	III	”

J, juvenile males; 0, light brown; I, darkened brown; II, light blue; III, dark blue.

of light. The size of the testis was measured during an exploratory laparotomy 8 days before the beginning of experimental exposure to light.

Of especial interest in Table II is the marked response of juvenile birds as compared with almost total lack of reactions in adult males which had previously passed through a breeding cycle. In the case of juvenile bird 78J, the lumina of the seminiferous tubules were lined with mature spermatozoa as early as 23 days after the beginning of light stimulation.

These results limit somewhat the importance of the light as a regulator of the yearly sex cycle in birds. In adult male sparrows an intrinsic sexual rhythm seems to be established which, through environmental factors, merely becomes synchronized with the seasons.

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[5] Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 484.

8610 C

Effect of Cattle Anterior Pituitary Extract on Bone and Cartilage of the Joint (Acromegalic arthropathia).

Martin Silberberg. (Introduced by Leo Loeb.)

From the Department of Pathology, Dalhousie University, Halifax, N. S., and the Department of Pathology, Santo Tomas Hospital, Panama, R. P.

In previous investigations[1, 2] we have established a stimulating influence of acid extract of cattle anterior pituitary gland on the growth of cartilage and bone of young guinea pigs. In the epiphyseal line, hypertrophy and hyperplasia of the various cell layers of cartilage took place. In the majority of cases, as a result of subsequent rapid calcification a premature closure of the epiphyseal line occurred, but in about 20% only proliferation of the cartilage cells was noticeable. This effect was likewise exerted on the cartilage of the chondrophyte. There was, therefore, good reason for assuming that, under the influence of the extract, growth of bone and cartilage of the joint might likewise be stimulated. Correspondingly, we wished to analyze the changes which take place within the joints.

The observations were made in the 30 guinea pigs which had also been used for the study of endochondral ossification under the influence of the extract. As to the technique, we refer to our previous paper.[1] In the present investigations, we studied the effects of the extract on the knee joint and on the upper part of the femur.

After 6 to 7, but still more pronouncedly after 10 injections, proliferation of the cartilage is seen, the first changes being observed within the transition cell zone: here the cells become hypertrophic and hyperplastic; more and more they become arranged in a longitudinal direction in contradistinction to their flat horizontal position under normal circumstances. After 14 injections these cells have become still more numerous, and at the same time they assume a distinct columnar arrangement, and are stained dark bluish with hematoxylin, as an indication that they are still relatively immature; the stroma between them is diminished in amount. The proliferation within the transitional zone extends gradually in the direction towards the sliding zone of cartilage on the surface of the joint. This proliferative process is followed by liquefaction and degeneration of the growing cartilage. Subsequently small vacuolar ridges are formed, and in later stages we may even find gaps within the rapidly growing zone of the cartilage. Finally, the surface of the joint is reached and here microscopic ulceration occurs. The pressure zone of the cartilage participates in these processes as well: Vascularization and ingrowth of bone-marrow into the cartilage cell layers penetrating up to the surface are noted. In addition, within the cartilage cell layers disturbances of calcification and of endochondral ossification are found.

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[1] Silberberg, M., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 1033.

[2] Silberberg, M., *ibid.*, 1936, **34**, 177.

After 21 injections, ulceration is seen especially in the neighborhood of the insertion of the ligaments. Here the covering of the surface of the cartilage by cells is comparatively thin; it is therefore to be expected that here also the most marked changes should take place, but to a less marked extent they are noted everywhere in the joint.

We may then conclude that the behavior of the cartilage of the joint corresponds to that observed within the chondrophyte and at the epiphyseal line. The growth phenomena in these 3 places (1) epiphyseal line, (2) chondrophyte, and (3) joint have to be considered as an entity. One may state the rule that the greater the proliferation of the cartilage, the more pronounced will be the ulceration of the surface of the joint and the less the calcification, the greater the tendency to ossification, the more extensive will be the calcification of the cartilage cells, and the less the ulceration.

Our experimental findings correspond in every respect to the "acromegalic arthritis" as described by Erdheim[3] at the "Knorpelknochengrenze." Inasmuch as in our experimentally produced conditions, any marked inflammatory reaction was missing, our findings would better be designated as "acromegalic arthropathia."

Conclusions. In young guinea pigs, under the influence of acid extract of cattle anterior pituitary, the growth-promoting effect manifests itself as acromegalic arthropathia in 2 directions: (1) Hypertrophy and hyperplasia starting in the transitional zone of cartilage then proceeding towards its sliding and pressure zone, and in a later stage leading to its liquefaction and to ulceration of the surface. (2) This stimulation of the growth of cartilage may be followed by calcification. In the latter case the ulcerative processes are less pronounced than in the former. Whether the one or the other of these 2 effects occurs does not depend upon the number of injections given to the animals.

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[3] Erdheim, J., *Veroeffentlich, a. d. Gebiete d. Pathol. in Einzeldarstellungen,* 1931, Julius Springer, Berlin.

8611 C

Effect of Preservatives on Undenatured Bacterial Antigens.*

A. P. Krueger and V. C. Nichols.

From the Department of Bacteriology, University of California, Berkeley.

In the preparation of undenatured bacterial antigens mass-cultures of recently isolated bacteria are washed free of metabolites and media-constituents with Locke's solution.[1] The cells are fragmented in a special ball-mill[2] and residual intact cells are removed by ultrafiltration.[3] Filtrates containing the chemically unaltered antigenic fractions of the organisms are standardized on the basis of native-protein content and are called Undenatured Bacterial Antigens or more briefly U B A. Antigens of this type have been tested clinically during the past several years.[4-13]

Since the major desideratum of the preparative technic has been avoidance of denaturation it became important to determine just how readily the antigens were altered when exposed to such degrees of heat or chemical action as might be encountered under practical working conditions. We have shown in the case of Staphylococcus U B A[14] that thermal denaturation begins to be significant at 40°C.; the reaction obeys the mass-law and exhibits the remarkably rapid rise in rate with increase in temperature that characterizes protein denaturation in general (critical thermal increment 44,000).

In order to test the adequacy of certain antiseptics to preserve U B A solutions without causing appreciable denaturation, phenol, tricresol and merthiolate were added to aliquots of freshly pre-

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* Supported by Grant-in-Aid of Research from Eli Lilly and Company.

[1] Krueger, A. P., *J. Infect. Dis.*, 1933, **53**, 237.

[2] Krueger, A. P., *J. Infect. Dis.*, 1933, **53**, 185.

[3] Krueger, A. P., and Ritter, R. C., *J. Gen. Physiol.*, 1930, **13**, 409.

[4] Krueger, A. P., Nichols, V. C., and Frawley, J. M., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1097.

[5] Frawley, J. M., Stallings, M., and Nichols, V. C., *J. Pediatrics,* 1934, **4**, 179.

[6] Frawley, J. M., *J. Pediatrics*, 1934, **4**, 184.

[7] Munns, G. F., and Aldrich, C. A., *J. Pediatrics*, 1934, **5**, 590.

[8] Stallings, M., and Nichols, V. C., *Am. J. Dis. of Child.*, 1934, **48**, 1183.

[9] Kracaw, F. C., *Calif. and West. Med.*, 1934, **40**, 228.

[10] Hosmer, M. N., *Calif. and West. Med.*, 1935, **43**, (July).

[11] Kracaw, F. C., *The Laryngoscope,* January, 1936.

[12] Womack, D. R., *The Laryngoscope,* July, 1935.

[13] Fuendeling, M. J., *Northwest Medicine* (In press).

[14] Krueger, A. P., and Nichols, V. C., *J. Bact.*, 1935, **30**, 401.

pared staphylococcal U B A in such amounts that the final concentrations were 0.3% tricresol, 0.5% phenol, 0.01%, 0.005%, and 0.002% merthiolate. The solutions were kept at room-temperature for 5 months during which time weekly samples were withdrawn for determination of their content of total, denatured, and native protein. The analytical methods have been described by Krueger and Nichols.[14]

A composite graph of the data obtained in 2 experiments is shown in Fig. 1. It is evident that within the first week at room-temperature the denaturing effect of the preservatives had reached a maximum and after this time equilibrium was established; no further denaturation occurred as long as the temperature was constant. Wide fluctuations from the curves shown in Fig. 1 were observed with a third lot of antigen which was allowed to stand at room-temperature during the summer of 1935. As the temperature rose the concentrations of native protein dropped and those of denatured protein rose. When the room temperature again became 22°C. the content of native protein increased at the expense of the denatured protein and equilibrium again became established at the values recorded in Fig. 1.

Merthiolate in concentrations of 1:50,000 or 1:20,000 produces

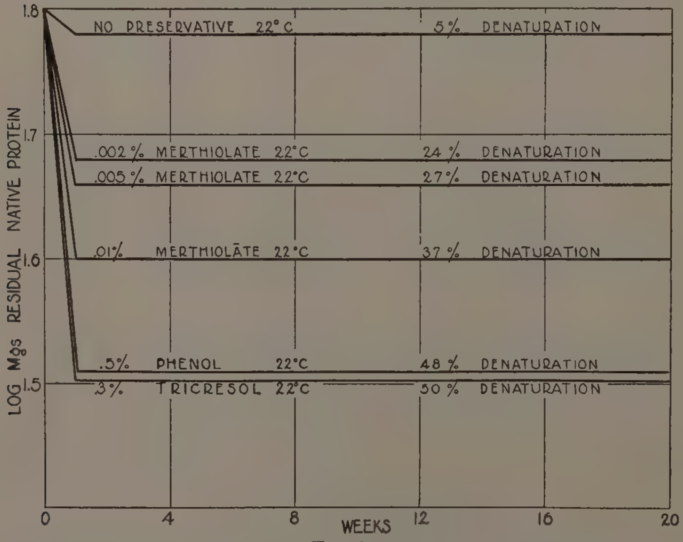

Fig. 1.

The Denaturing Effect of Antiseptics on Staphylococcal Undenatured Antigen.

considerably less denaturation than phenol or tricresol, in the concentrations ordinarily employed for vaccines. It is of practical significance that summer temperatures will shift the equilibrium between native and denatured fractions in favor of the latter, this shift appears to be reversible and the concentration of native protein increases when the temperature is lowered.

8612 P

Pure Monophasic Action Potentials and Their Employment in Studies of Ventricular Surface Negativity.

Harold C. Wiggers. (Introduced by C. J. Wiggers.)
From the Department of Physiology, Western Reserve University Medical School, Cleveland, Ohio.

Recent interpretations of monophasic action potentials led from an injured and uninjured spot on the ventricular surface hold (1) that the entire deflection is dominated by electrical potential alterations at the *uninjured* spot,[1] (2) that it is essentially influenced by potential variations at the *injured* spot,[2] or even more specifically, (3) that the initial rise may be governed by electrical variations at the injured region, whereas its rapid offset is determined by the discontinuance of negativity at the normal region.[3] Further experimentation makes it apparent that correct interpretations have been hampered by registration of impure monophasic waves, *i. e.,* curves that exhibit oscillations or notches either before the rise or somewhere on the ascending limb, or which give evidence of auricular and other extraneous activity. Previous investigators have been obliged to apologize for inescapable extrinsic effects and to devise explanations to account for them. By recording from a discrete spot of injury, as produced by a new type of suction Ag-$AgCl_2$ electrode devised by the author, and another Ag-$AgCl_2$ wick electrode in apposition with the epicardial surface, it proved possible to record consistently monophasic curves that rise acutely from a straight base line and which exhibit no vibrations on the upstroke. An example is displayed in Fig. 1.

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[1] Jochim, K., Katz, L. N., and Mayne, Walter, *Am. J. Physiol.,* 1934, **111**, 177.

[2] Wilson, F., Macleod and Johnston, Proc. Soc. Exp. Biol. and Med., 1933, **30**, 797.

[3] Wiggers, H. C., and Wiggers, C. J., *Am. J. Physiol.,* 1935, **113**, 683.

The first purpose of this preliminary communication is to present some evidence that such perfect curves are not influenced by potential changes of the injured area, whereas the so-called extrinsic effects, which appear in the form of notches on the ascending limbs of impure curves are mainly caused by such alterations.

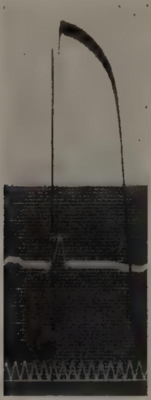

FIG. 1.
Pure monophasic curve from ventricle (large curve) and E.C.G. (lead II). Time, .02 sec.

If monophasic curves from an injured-uninjured region are recorded on paper travelling 100-150 mm./sec. in relation to the waves of a Lead II standard E.C.G., and immediately thereafter monophasic records are taken from the same injured spot and a leg, after the manner of Wilson *et al.*,* then the incidence of the rise of 2 such curves should coincide in all cases regardless of the location of the injured spot, as shown in diagram I of Fig. 2. Actual curves have shown that, as illustrated in diagrams II and III, the rise of the pure monophasic curves either comes earlier or later than the initial deviation in unipolar-leg leads. When, however through imperfections of technique of leading, chiefly at the injury electrode, oscillations precede or are superimposed upon the ascending limb, their incidence is usually identical with that of the polarity changes at the injured spot as exemplified by the dotted line curves in diagrams II and III. In cases where records indicate the 2 rises as coinciding, the ascending limbs always possess these notches, as in diagram II. When the true monophasic curve actually begins ahead of the unipolar curve, the effects of this positivity under the injured electrode are seen on impure curves by a sudden sharp upward gradient on the ascending limb, depicted by the dotted oscillations in diagram III. If positivity and negativity should coincide at the rise, then this curve could still be looked upon to signify the incidence of negativity under the uninjured electrode lead.

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* There are theoretical objections to simultaneous leads from the same injured area.

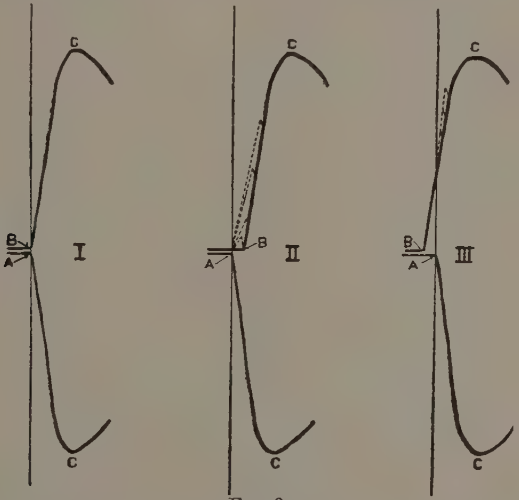

FIG. 2.

Three diagrams illustrating relations of pure and impure monophasic curve (upper) to unipolar leads from point of injury and left leg (lower). Discussion in text.

It is the second purpose to report that when pure monophasic curves are recorded from various points on the anterior and posterior surfaces of the ventricles, the point of injury being kept constant, then most points become negative at practically the same moment. This more nearly confirms the original descriptions of Erfmann[4] and Clement[5] than those subsequently, submitted by Lewis and Rothschild[6] as a result of unipolar studies.

Attention is called to 3 interesting points: (1) A calculation of the maximum time-difference between the earliest and the most retarded appearance of negativity on the right and left heart in a series of 10 experiments, if the region overlying the interventricular septum be excluded, reveals that in no case is this interval greater than 5 sigma, which is within the limit of the error of measurement. (2) The right ventricular surface receives its excitation only 5 sigma earlier than that of the left, the error of measurement having been taken into account. This time-difference is measured in sigma, not in hundredths of a second as were those of Lewis and Rothschild. (3) In over 80% of the cases, the epicardial surface overlying the interventricular septum apparently receives its excitation very definitely ahead of the remaining right and left myocardial surfaces, in some instances to the extent of 17.5 sigma.

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[4] Erfmann, W., *Z. f. Biol.*, 1913, **61**, 155.

[5] Clement, E., *Z. f. Biol.*, 1912, **58**, 110.

[6] Lewis, T., and Rothschild, M. A., *Phil. Trans. Roy. Soc. London*, 1915, B 206, 181.

The results now reported suggest that the impulses spread more rapidly than generally stated through the myocardial tissue so as to reach all points of the anterior and posterior surfaces practically simultaneously. A more thorough reinvestigation of the velocity as well as the spread of the cardiac impulses seems to be indicated.

8613 P

A Rat Test for Drug Addiction.*

E. J. STANTON. (Introduced by A. R. Moritz.)

From the Department of Pharmacology of the School of Medicine, Western Reserve University, Cleveland, Ohio.

Further experiments on the addiction liability of various drugs in the albino rat have been carried out by the method previously described.[1] The test measures objectively progressive changes in the degree of irritability of the animal 24 hours after withdrawal of the drug throughout the period of administration. Advantage is thus taken of the most outstanding part of the abstinence syndrome clinically and experimentally, hyperirritability, to assess the ability of a drug to produce addiction.

The irritability of the rat which is normally fairly constant for a series of animals is quantitatively measured by determining the struggle response to a uniformly uncomfortable situation. Such a situation is provided by lashing the animal in the supine position to a small animal board so constructed that the animal is free to

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* A part of this investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[1] Himmelsbach, Gerlach, Stanton, *J. Pharm. Exp. Therap.*, 1935, **53**, 179.

struggle but can neither injure nor extricate itself. Graphic records of each struggle are made kymographically by a light lever attached to the skin overlying the xyphoid process. The response of the animal is expressed by the number of struggles per interval and is a quantitative index of the degree of irritability of the animal at that time.

Daily administration of morphine, codeine, or heroine results consistently in progressive increase of 24-hour abstinence hyperirritability as shown by the struggle response to the above described uniformly uncomfortable situation.[1] Control animals receiving injections of water showed only a gradual decrease in irritability under the same experimental conditions.

Dihydromorphinone hydrochloride (dilaudid): To completely eliminate the movements of rats in the device described above, dilaudid was 10 times as potent as morphine. Complete tranquilization was obtained with dilaudid in one-tenth of the dosage necessary with morphine. When daily doses of equal tranquilizing potency were given, no significant difference in addiction liability could be found between dilaudid and morphine. Tolerance, judged by response to the same uncomfortable situation, one hour after injection, developed to the same degree in daily administration of both morphine and dilaudid. Likewise, no difference in the duration of hyperirritability upon the abrupt and complete withdrawal of the drugs was found.

Barbiturates: Two barbiturates were administered subcutaneously daily for a period of 7 weeks. Pentobarbital-sodium, a short-acting member of this series, was given in 30, 15, and 5% M.F.D. daily while phenobarbital-sodium, a long-acting barbiturate with marked cumulative action, was administered at 15 and 5% M.F.D. daily. No increase in abstinence irritability to either drug was found. On the contrary, irritability decreased progressively below the control level indicating some cumulation of depressive effect of both drugs. Only a minor degree of tolerance to the maximal effects was indicated by the struggle response of the rats one hour after injection but the duration of somnifacient action of pentobarbital appeared by direct observation to be markedly shortened at the end of the experimental period.

Acetanilid: Clinical reports of acetanilid addiction as well as the report of Payne[2] of the production of abstinence symptoms in dogs suggested its inclusion in this study of addiction in rats.

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[2] Payne, *J. Pharm. Exp. Therap.*, 1935, **53**, 401.

A 2% suspension of the drug in 2% gum acacia was administered daily by stomach tube in doses of 50, 100, and 200 mg. per kg. for a period of 3 months. Such doses are large even for the rat, being respectively 4, 8, and 16 minimal effective doses (M.E.D. in yeast fevered rats, 12.5 mg. per kg.[3]). No evidence of addiction was found; the 24-hour withdrawal irritability closely followed that of the controls receiving 2% gum acacia solution alone.

Experimental evaluation of insulin in the treatment of morphine abstinence hyperirritability: Several favorable clinical reports of the efficiency of insulin in relieving the symptoms of morphine withdrawal[4, 5] suggested a trial of its ability to relieve abstinence hyperirritability in rats.

The 24-hour withdrawal hyperirritability of rats addicted for periods of 4 to 5 weeks on ascending doses of morphine sulphate was treated with graded doses of insulin and the struggle response before and after treatment compared. The results were inconstant at various doses but median results were about the same as those produced by the injection of tap water.

An attempt was also made to alter the course of addiction and tolerance by the daily administration of insulin. One hour before the daily dose of morphine, insulin was injected in a dosage shown to produce a marked (but in no case fatal) hypoglycemia. No effect on either addiction or tolerance could be shown; addiction developed at the same rate and to the same degree in animals receiving morphine-insulin as in those receiving morphine-tap water. The rate and magnitude of tolerance development was likewise essentially identical in the 2 groups.

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[3] Smith and Hambourger, *J. Pharm. Exp. Therap.*, 1935, **54**, 346.

[4] Sakel, *Ztschr. f. d. ges. Neurol. u. Psycho.*, **129**, 639; *ibid.*, **143**, 506.

[5] Braun, *Schweiz. Arch. f. Neurol. u. Psych.*, **27**, 226.

Minnesota Section

University of Minnesota, March, 18, 1936.

8614 C

Precipitin Reactions with Bone and Teeth.

Arthur T. Henrici.

From the Department of Bacteriology and Immunology, University of Minnesota.

It was suggested by Professor A. E. Jenks of this University that immunological reactions might be used to determine the origin of archæologic relics composed of bone or ivory. To test the feasibility of this project, the following experiment was undertaken.

A fox's skull was roughly cleaned of meat, and the brain was washed out. It was placed on a roof and exposed to the weather for 2 weeks. During this time it became covered with maggots which dried up and dropped off. The skull was then thoroughly scrubbed, rid of practically all adherent soft tissue. Nearly all the teeth dropped out during this operation, and the remainder were extracted. The skull and teeth were again exposed to the weather for 3 weeks, after which they were kept indoors for an additional 2 months.

Four rabbits were injected with fox-serum twice weekly for 12 weeks. The pooled serums precipitated with fox-serum in dilutions up to 1:500,000.

The skull was wrapped in a towel and crushed. The crushed bone was ground to a rather coarse powder, the largest particles being about 0.5 mm. in diameter. The teeth were ground separately. Both bone and teeth were then extracted with a volume of salt-solution slightly less than the volume of ground material, for 2 hours at room-temperature, shaken repeatedly, and kept over night in the ice-box. Precipitin-tests with these extracts were completely negative with the teeth, and very faintly positive with the bone, the reaction with the latter being about the same in degree as with fox-serum diluted 1:100,000.

This experiment indicates that the use of the precipitin-test in identifying archæologic relics, while not absolutely hopeless, is not very promising.

8615 P

Variation of *Micrococcus tetragenus.*

HOBART A. REIMANN.

From the University Hospital, Minneapolis.

Numerous recent studies suggest that the phenomenon of microbic dissociation or dissociative variation is exceedingly complex and involves more than the M, S, R, and G phases so extensively studied during the past decade. Evidence at present indicates that in addition to these so-called phase-transformations, another form of variation exists which involves even greater biologic differences. In studies on a strain of *Micrococcus tetragenus* obtained from a patient I have encountered more than 13 different forms and have isolated and studied 10 of the fairly stable ones.[1] They were named according to pigment-production, colonial morphology and cell-morphology as follows: mucoid-yellow, yellow, mucoid-white, white, mucoid-pink, pink, pink-yellow, brown, translucent, and a bacillary form. There was evidence of frequent change from one form to another and each of the forms except the bacillary one was related to the other directly or indirectly by antigenic similarity. In attempting to correlate the 10 different forms with the M, S, R, and G forms of other bacteria, difficulty was encountered in the fact that 3 mucoid forms existed in the same presumed dissociative pattern. Furthermore no true R forms had been noted during 18 months of observation. The recent appearance of distinctly rough, dry, crinkled, and adherent pink colonies among smooth pink ones when an 8-month-old-broth-culture of the latter form was plated, suggested the possibility that at least 2 kinds of bacterial variation exist. The white, yellow, pink, pink-yellow, and brown forms may represent distinct variants or may even be regarded as antigenically specific types analogous to those existing, for example, among pneumococci, whereas the mucoid-white, mucoid-yellow, mucoid-pink, and rough-pink forms may represent cultural phases of the respective types. The place of the translucent form and its occasional lytic colonies in the scheme has not yet been determined. Thus far I have isolated the usual set of dissociant forms M, S, and R of only the pink type. The bacillary form derived from the pink on numerous occasions may represent the G form. No reversion of the bacillary form to the pink coccic form occurred in the experiments but has been observed by others.[2] The following scheme represents the types (variant forms) and cultural phases of the types thus far isolated:

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[1] Reimann, H. A., *J. Bact.*, April, 1936 (In press).

Culture phases	TYPES.					
M	Mucoid-yellow	Mucoid-white	Mucoid-pink			
S	Yellow	White	Pink	Pink-yellow	Brown	Translucent
R			Rough pink			?Translucent lytic colonies
G			?Bacillary form			

Search is at present underway to detect and isolate the M forms of the pink-yellow and brown types and the R forms of the yellow, white, pink-yellow, and brown types, if they exist.

Of further interest is the evidence of specific antigenic parallelism among strains obtained from various sources.[1] For example, the yellow, white, and pink forms of the strain under study were, among themselves, antigenically distinct, but each was similar to the corresponding forms of 2 of 10 other strains of *M. tetragenus*. This again suggests a type-specificity among strains of *M. tetragenus* akin to that existing among pneumococci, and lends indirect support to the theory of the interchangeability of types of other bacteria. It is well known that pneumococci can be changed from one type into another and that each type has cultural phases of M, S, and R forms.[3]

These findings in general conform in a remarkable way with the hypothesis of Hadley concerning different categories of variability which will be published in a forthcoming paper.[4]

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[2] Schmidt-Kehl, L., *Arch. Hyg.*, 1930, **103**, 235.

[3] Griffith, F., *J. Hyg.*, 1928, **27**, 113; Reimann, H. A., *J. Exp. Med.*, 1929, **49**, 237.

[4] Personal communication to the author.

New York Meeting

New York Academy of Medicine, April 15, 1936.

8616 C

A Simple Method for Determination of Ethyl Alcohol in Blood.

JULIUS C. ABELS. (Introduced by I. Greenwald.)

From the Department of Chemistry, New York University Medical College.

While methods for the determination of ethyl alcohol in blood have been known for many years, all those sufficiently accurate for clinical or medico-legal purposes involve specialized apparatus or difficult techniques. A simple, rapid method of estimating the small amounts of blood alcohol in normal and intoxicated individuals has long been needed. The procedure that we propose is a combination of those of Widmarck[1] and Heise[2] with certain modifications. We have avoided the use of Widmarck's carefully designed desiccating apparatus, as well as his final oxidimetric titration which requires a certain amount of chemical technique, and is, besides, time consuming; we employ only his principle of isolating the alcohol from the blood by desiccation over concentrated sulfuric acid containing potassium dichromate, and this we have much simplified by using rolls of filter paper and ordinary flasks (see below). We have likewise disposed of the initial blood distillation proposed by Heise which has never worked successfully in our laboratory; his final colorimetric estimation of the EtOH by comparing a known amount of dichromate-sulfuric acid solution reduced by it with a set of standards is all that is retained.

In short, the principle of the proposed procedure is to absorb the alcohol from blood caught on a roll of filter paper into a solution of potassium dichromate in concentrated sulfuric acid. This solution is reduced and develops a green or blue color which is finally compared with a set of standard solutions made from known amounts of alcohol.

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[1] Widmarck, E., *Bioch. Z.*, 1922, **131**, 473.

[2] Heise, F., *Am. J. Clin. Path.*, 1934, **4**, 14.

One ml. of 0.33% potassium dichromate solution in sulfuric acid (made by dissolving 333 mg. dichromate in one ml. of water, and diluting to 100 ml. with concentrated sulfuric acid) is spread on the bottom of a 50 ml. Erlenmeyer flask. 0.5 ml. of blood are then pipetted onto a roll of filter paper prepared according to Fig. 1.

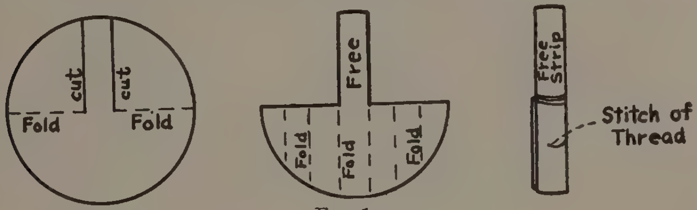

FIG. 1.

The long strip of paper left attached to the roll is inserted between the cork and neck of the flask, thus allowing the absorbed blood to be suspended over the dichromate solution. After being heated from 15 to 30 minutes in a boiling water bath or drying oven at 100°C., the flask is cooled, the cork and wad of paper are removed, and 1.7 ml. of water are added, bringing the total volume of the contents to 3.0 ml. (one ml. dichromate solution, close to 0.3 ml. water from the desiccated blood, and 1.7 mls. water now). The diluted solution is poured into a test tube (6″ x ½″) and its color finally compared with the standard set.

Fifteen standards are made containing 0, 0.05, 0.10, . . . 0.70 mg. EtOH in 3 ml. total volume from 0.01% and 0.05% EtOH stock solutions, water, and one ml. of the dichromate-sulfuric acid solution. These standard systems are heated 10 minutes in a boiling water bath, and the test-tubes (6″ x ½″) are then tightly corked and sealed. If they are exposed as little as possible to the light, they keep for at least 2 weeks.

Thirty determinations using water alone and all the types of filter paper in our laboratory failed to show any dichromate reduction. Applying the method to the determination of alcohol in the bloods of various animals, we obtained the values in Table I.

TABLE I.

Animal	No. observations	% EtOH
man	40	0.000-0.010
pig	3	0.005-0.020
dog	3	0.005-0.010
ox	3	0.010

Experiments were also made on samples of ox blood to which various amounts of alcohol were added. The results of these are shown in Table II.

TABLE II.

No. experiments	mg. EtOH added to 100 ml. blood	EtOH found (average)	Aver. blank	Recovery %
30	0.100	0.104±.004	0.010	94
10	0.200	0.204±.007	0.010	97
10	0.300	0.290±.017	0.010	93

It is quite necessary to use freshly drawn blood in this procedure, for we have found as much as 25% of the EtOH to be destroyed upon keeping the blood sample in the ice chest for 24 hours, and more than 50% after 72 hours.

Micro method: The above method has been extended to determine similar concentrations of EtOH in as little as 0.05 ml. of blood. This amount can be easily obtained from a single finger puncture; a 1% solution of picric acid is employed for sterilizing the skin.

The principle of the method is the same as above, and except for 2 minor changes, the technique is unaltered. 0.20 ml. of the dichromate-sulfuric acid solution are pipetted onto the bottom of a flask such as shown in Fig. 2; the finer the pipette is, the less chance is there of smearing any of the oxidizing solution on the inside of the neck of the flask, with consequent reduction by the filter paper when later introduced. This source of error must, of

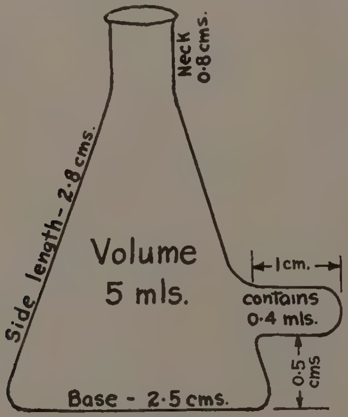

Fig. 2.

course, be avoided, and can be by using a fine pipette. 0.05 ml. of blood are absorbed onto a roll of filter paper (Whatman's hardened No. 5, 1″ diameter) which is suspended over the dichromate solution as in the macro method. The flask is then heated in a boiling water bath or drying oven at 100°C. for 20 minutes, cooled, the cork and paper removed, and the contents of the flask finally diluted with 0.07 ml. of water, bringing the total volume to 0.3 ml. The flask is tipped on its side allowing the contents to flow into the side arm; and the color of this resulting solution is matched against a series of standards contained in small test tubes with an internal bore and wall thickness the same as those of the flask projections.

Eight standards are made containing 0, 0.20, 0.40, 1.40 mg. EtOH in 3 ml. total volume from 0.20% EtOH stock solution, water, and 2.0 ml. of the dichromate sulfuric acid solution. The systems are heated for 10 minutes in a boiling water bath, and 0.3 ml. of each are pipetted into the small test tubes which are immediately sealed.

Twenty-five determinations, using water alone on the filter paper, failed to reveal any reduction of the dichromate solution as long as a hardened filter paper was used. Soft papers are to be avoided, for in dealing with so small a volume of oxidizing mixture, a small thread of paper falling into it will invalidate the results of an experiment. Thirty-two determinations on human blood placed the EtOH concentration of human blood very near 0.00%, and certainly under 0.025%. Forty experiments on artificially prepared bloods containing from 0.10% to 0.30% EtOH gave, after subtracting the blanks, recovery values of from 100% to 107%.

We have also been able to apply both methods to a study of the blood alcohol concentrations during various stages of intoxication, and have found the two to agree within ± 3%. This latter study was undertaken in order to correlate the symptomology of such stages with the corresponding amounts of EtOH in the blood. We divided the cases into 4 groups, as:

I. Chronic alcoholics then sober; II. mildly intoxicated individuals; III. markedly intoxicated individuals; and IV. individuals showing clouding of consciousness (semi-stuperous to comatose). The results of these clinical experiments are perhaps best expressed in Fig. 3.

Although this method really measures the reducing substances in the blood volatile at 100°-105°C., the amount of these other than

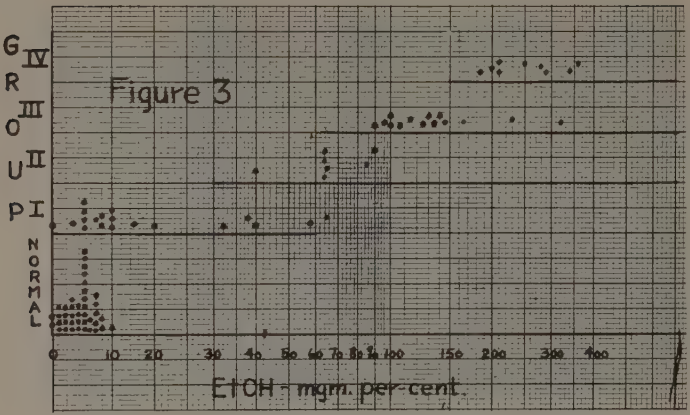

FIG. 3.

ethyl alcohol is normally small; acetone, the second largest fraction, constitutes about 20% of the total.[3] In diabetic coma, however, the amount of free acetone may reach 25 mg. %,[3] but there can be no danger of diagnosing this as an alcoholic coma where the "reducing power" of the blood is at least 8 times this value. We have run a determination on the blood of a patient in terminal diabetic ketosis, and have found the sample to possess a reducing power equivalent to only 0.015% EtOH.

Paraldehyde, a substance much used in the treatment of alcoholics, also does not interfere with the results. Bloods of patients in comas from paraldehyde poisoning show normal alcohol values.

Small mistakes in dilution in the macro method introduce no significant error, for what is really measured is the quality of the resulting solution. The macro method lends itself better to a quantitative study of alcohol in the blood, as might be required in problems on its metabolism; the micro method is sufficiently convenient and accurate for clinical diagnoses of alcoholism.

I wish to express my gratitude to Dr. I. Greenwald for having suggested this work, and for his very kind interest in, and many helpful suggestions to the problem. I also am indebted to Dr. R. K. Cannan and Dr. N. Jolliffe for the ready cooperation they extended to me at all times.

Summary: A method is presented for the determination of EtOH in blood. We have applied this method to the estimation of alcohol in the bloods of various animals and the bloods of men in different stages of intoxication. This procedure, we believe, should prove useful in many problems involving alcoholic intoxication.

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[3] Marriott, W. M., *J. B. C.*, 1914, **18**, 507.

8617 P

Hemorrhagic Necrotic Skin Lesions in Rabbit Produced by *Hemophilus influenzæ* and *Hemophilus pertussis*.*

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When injected into the skin of rabbits, cultures of *H. influenzæ* can readily be distinguished from cultures of *H. pertussis*. While *H. influenzæ* causes slight inflammation, characterized by redness and swelling, *H. pertussis* produces bluish-violet discoloration in the involved part of the skin, frequently followed by necrosis.[1, 2] The observations to be reported are concerned with the influence of intravenous injection of *H. influenzæ* and *H. pertussis* on the skin lesions caused by the intradermal injection of the respective organisms.

For our experiments *H. influenzæ* is cultivated on Levinthal agar, *H. pertussis* on Bordet-Gengou medium, containing 25% rabbit blood. Twenty-four or 48-hour cultures of *H. influenzæ* and 48-hour cultures of *H. pertussis* are suspended in saline. The bacterial content of the suspension varies between 2 to 4 billion microbes per cc. Rabbits are injected intradermally with 0.25 cc. each of suspensions of *H. influenzæ* and *H. pertussis* and reinjected intravenously 24 hours later with 1 to 2 cc. of a suspension of *H. influenzæ*. Three to 6 hours later the areas prepared intradermally with *H. influenzæ* are transformed into a bluish-black lesion, and necrosis follows. The influence of the intravenous injection on areas inoculated with *H. pertussis* is only moderate, mostly none. The intravenous injection of *H. pertussis* produces the same results as the intravenous injection of *H. influenzæ*. It also may lead to hemorrhagic-necrotic lesions in the areas of the skin previously inoculated with *H. influenzæ*, while the areas inoculated with *H. pertussis* are usually not affected. We have the

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* Aided by a Grant of the Colonel Friedsam Foundation Fund.

[1] Takagi, Y., cited by Kasahara, M., *Klin. Wochenschr.*, 1933, 1609.

[2] Gundel, M., and Schlueter, W., *Z. Blatt for Bact.*, Orig. 1933, **129**, 461.

impression, however, that the intravenous injection of *H. influenzæ* is more powerful than the injection of *H. pertussis*. It should be mentioned that in these experiments about one-third of the rabbits employed proved to be refractory.

TABLE I.
Influence of Intravenous Injection of *H. influenzæ* on Skin Areas 24 Hours Previously Injected with *H. pertussis* and *H. influenzæ*.

Aspect of Skin Areas	Rabbit skin inoculated with I. *H. pertussis*	 II. *H. influenzæ*
A. Before intravenous injection	Bluish-violet lesion with indistinct outline	Redness, sometimes slight swelling with a tiny yellowish spot in the center
B. 5 hours after intravenous injection	No change	Dark bluish discoloration, distinct outline, beginning necrosis
C. 24 hours after intravenous injection	Appearance of small hemorrhagic - necrotic areas within or around the bluish-violet lesions	Hemorrhagic-necrotic lesions with distinct outline

According to Shwartzman, he and Frisch obtained an exotoxin from *H. influenzæ,* as did Gross, Mishulow, Mowry and Scott and later Koplik from *H. pertussis.*[3, 4, 5, 6, 7] We also succeeded in preparing effective agar-washing filtrates of *H. influenzæ* using the technique described by Shwartzman for the preparation of *B. typhosus* agar-washing filtrates. It seems however, unlikely that the skin lesions described in this paper can be attributed to exotoxins. Suspensions of *H. influenzæ* washed several times were equally able to produce the hemorrhagic-necrotic reactions while the corresponding wash waters and Berkefeld filtrates of the supernatant fluids were lacking in this ability. Heat-killed *H. influenzæ* were also able to induce hemorrhagic-necrotic skin lesions under the same experimental conditions as did living *H. influenzæ.* Thus the active principle must be connected with the bacteria themselves.

There exists, however, an interesting relationship between the agar-washing filtrates of *B. typhosus* and *meningococcus,*[†] on the one hand, and the effectiveness of *H. influenzæ* in the skin of the rabbit, on the other.

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[3] Shwartzman, G., *Klin. Wochenschr.*, 1930, p. 1925.

[4] Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 581.

[5] Mishulow, L., Mowry, I. W., and Scott, E. B., *J. Immunol.*, 1930, **19**, 227.

[6] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 843.

[7] Koplik, L. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 309.

[†] We are grateful to Dr. Shwartzman for providing us with highly effective preparations of *B. typhosus* and *meningococcus* agar-washing filtrates.

B. typhosus agar-washing filtrates were injected intravenously into rabbits which 24 hours previously had received intradermal injections of *H. influenzæ* and *H. pertussis.* Three to 6 hours following the intravenous injection the areas of the skin treated with *H. influenzæ* became bluish-black, but there was no distinct change to be observed in the areas inoculated with *H. pertussis.* Conversely, living as well as dead *H. influenzæ* if injected intravenously also activate areas intradermally prepared with *B. typhosus* agar-washing filtrates.

The results of the above-mentioned experiments show that *H. influenzæ* may, under certain conditions, produce a hemorrhagic-necrotic lesion in the skin of rabbits. This ability is certainly not a specific characteristic of *H. influenzæ* alone. However, other organisms related to infections of the upper respiratory tract, such as pneumococci and streptococci, were not able to produce an identical effect under the same experimental condition as easily as did *H. influenzæ.*

8618 P

Subcutaneous Temperatures in Localized Infections.

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The mechanism of the increased production of heat at the site of a localized infection in the human body never has been definitely understood. The theory that the production of heat around an ordinary localized inflammation is due to the increased rate of flow of blood at the site has been upheld by the experimental studies of Marchand.[1] The belief that the local increase in temperature is due to heat-producing chemical processes active at the site of infection and that the hyperemia is really a compensatory phenomenon has been promoted by the work of Segale,[2] Schade,[3] Gessler[4] and others.

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[1] Krehl, Ludolf, and Marchand, F., *Handbuch d. allgem. Path.*, 1924, Vol. 4, Part I, S. Hirzel.

[2] Segale, M., *J. Exp. Med.*, 1919, **29**, 235.

[3] Schade, H., *Die Physik. Chemie in d. inneren Medizin*, 1923, 3rd edition, Steinkopff, Dresden and Leipzig.

[4] Gessler, H., *Arch. f. exp. Path. u. Pharm.*, 1921, **91**, 366.

Coincident with a study on experimental animals,[5] a series of clinical infections in human beings has been studied with regard to the variation in temperature in different areas of the same infection. The carbuncle was selected as a type of localized infection which commonly exhibits fairly constant pathologic changes. With regard to its blood supply, a carbuncle may be divided into 4 zones as shown in the accompanying diagram (see Fig. 1) as follows: Zone A, the area of central necrosis; Zone B, the area of bluish induration immediately surrounding Zone A; Zone C, the indurated,

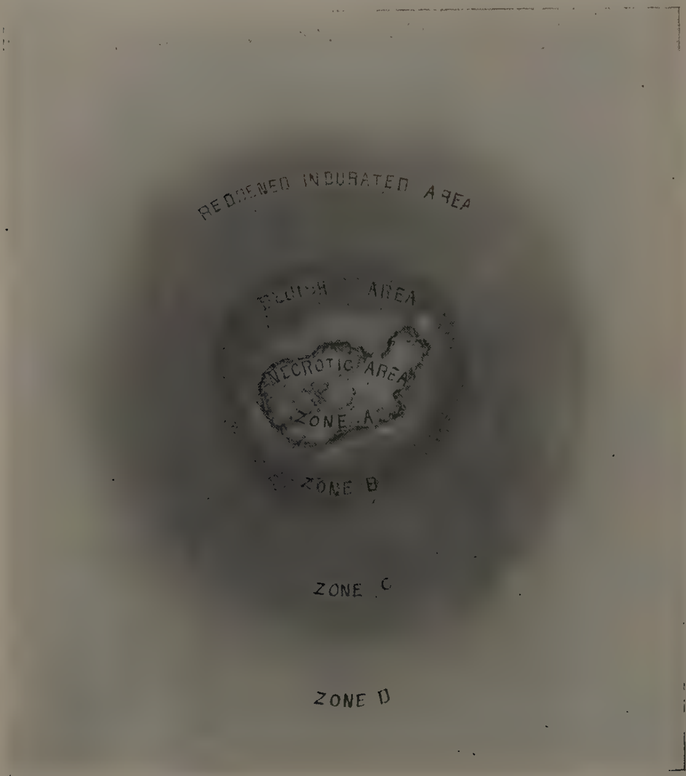

Fig. 1.

Artist's sketch of a carbuncle showing the division into zones for regional temperature observations.

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[5] Heuer, George J., Conway, J. Herbert, and Pickworth, M. E., Reported at a meeting of the New York Surgical Society, held at New York Hospital, May, 1933.

TABLE I.

Name of Patient	Duration of Lesion, days	Site of Lesion	Size of Lesion, cm. diam.	Leukocyte Count	Rectal Temp.	Normal Area	Zone A	Zone B	Zone C	Zone D
J.S.	4	Back of neck	3	14,500	37.8°C	37.4°C	36.8°C	37.4°C	37.8°C	37.4°C
A.R.	7	Over scapula	4	12,100	37.6	37.0	37.6	37.6	37.6	37.2
M.K.	4	Back of neck	5	7,900	37.9	36.7	37.6	37.3	37.6	36.7
G.H.	4	,, ,, ,,	6	19,000	37.8	37.8	36.0	37.3	37.9	37.3
C.U.	7	,, ,, ,,	4	10,100	37.8	37.4	37.4	37.7	37.7	37.4
M.K.	10	Over scapula	10	19,000	37.6	37.0	37.8	37.6	37.5	37.3
A.R.	4	Dorsal thorax	6	19,600	38.0	36.2	37.6	38.1	38.2	37.8
H.R.	2	,, ,,	4	11,400	37.8	37.0	37.0	37.6	37.6	37.4
J.W.	8	Back of neck	4	9,800	38.0	36.9	36.8	37.6	37.6	37.4
B.T.	8	,, ,, ,,	6	13,000	37.6	36.8	37.0	37.4	37.4	37.0
J.H.	14	Face	4	32,000	37.6	36.8	37.1	37.4	37.6	37.4
L.P.	7	Back of neck	6	10,900	38.1	37.0	36.6	37.8	38.1	37.8
A.B.	4	Forearm	6	10,600	38.4	37.2	37.2	38.2	38.2	37.8
A.H.	14	Face	6	17,000	38.8	37.4	36.8	38.2	38.6	37.0
A.T.	7	Back of neck	4	15,000	38.4	37.6	37.2	38.0	38.2	37.4
M.R.	14	Mastoid region	4	9,200	37.8	37.2	37.0	37.6	37.8	37.2
G.O.	10	Back of neck	6	19,000	38.0	37.2	37.0	37.6	37.9	37.4
J.C.	14	Wrist	6	10,300	37.8	36.8	36.5	37.4	37.4	37.0
J.S.	4	Chin	3	10,800	38.0	36.8	36.8	37.6	37.7	37.4
A.M.	14	Back of neck	4	12,800	38.0	37.7	37.2	38.0	38.0	37.7

acutely inflamed area surrounding Zone B (over this area the skin is usually markedly reddened and there is obvious increase in local heat); Zone D, the apparently normal area surrounding the carbuncle. In Zone D, there is no change in the color of the skin, no induration and no palpable increase in temperature.

From a consideration of the pathologic changes occurring in a carbuncle during the acute stage of the staphylococcus infection, the volume of blood should approximate normal in Zone D; should be maximal in Zone C; increased above normal in Zone B; and minimal or absent in Zone A. In the study of the 20 cases of carbuncle here recorded (Table I), temperatures were taken in each of the 4 zones referred to above, as well as in the subcutaneous tissue of a distant area of the body and in the rectum. Rectal temperatures were taken with mercury thermometers graduated to one-tenth degree centigrade and previously standardized with the needle-style thermocouple used for this study.* In each case the age of the patient, the duration of the carbuncle and the size and location of the lesion were noted. The room-temperatures were recorded; these showed slight variations which apparently had no effect on the relative temperatures in the different zones of the infected area.

A study of the table shows that the rectal temperature of the patient was consistently as high as or higher than any of the temperatures within the carbuncle itself. This finding would speak against the theory of Segale,[2] Schade[3] and Gessler.[4] In favor of Marchand's[1] belief there is the fact that, of the temperatures in Zones A, B, C and D, those in Zone C (where the hyperemia is maximal) regularly have been the highest. The temperatures have been consistently lowest in Zone A, the area of central necrosis, where the circulation of blood is minimal or absent.

The temperature-readings have been strikingly uniform, despite the fact that the virulence of the infection, the age of the patient, the size and location of the lesion, the rectal temperature and the leukocyte count have varied.

Summary. Carbuncles occurring in 20 different patients have been studied with reference to the local heat produced at the site of the infection. The temperatures, recorded by means of a needle-type thermocouple, have been listed for the various zones of a carbuncle. Certain findings have been strikingly uniform; they are as follows:

1. The temperature within the most acutely inflamed portion

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* Leeds and Northrup Company, Philadelphia, Pa.

of the carbuncle has rarely exceeded the rectal temperature of the patient at the same time.

2. The temperatures in the arbitrary zones established within a carbuncle on a basis of vascularity have, in general, been highest in the zone showing greatest evidence of active hyperemia and lowest in that area in which the tissue is necrotic and therefore ischemic.

3. The temperatures in the various zones of a carbuncle have shown a rather constant relation to each other and to the rectal temperature of the patient, despite variations in the febrile response of the individual to the infection, the age of the patient and the size and location of the lesion.

4. The temperatures of the apparently normal tissues directly surrounding the carbuncle have consistently been higher than those of the subcutaneous tissues at a distant point although that area showed no palpable or visible evidence of increased local heat.

These observations have been made as part of a series of experiments which, it is hoped, may throw some light on the nature of the mechanism active in the production of increased heat at the site of a localized infection.

8619 C

Cultivation of Poliomyelitis Virus *in vitro* in Human Embryonic Nervous Tissue.

Albert B. Sabin and Peter K. Olitsky.

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The current opinion of many investigators is that there is no unequivocal evidence that the virus of poliomyelitis has as yet been successfully cultivated outside the body. The recent demonstration that the virus is of very minute size (8 to 12 $m\mu^1$; 12 to 17 $m\mu^2$) emphasized the improbability that certain minute, visible microorganisms, which have been cultivated from poliomyelitic tissue, are etiologically related to the disease, while a critical analysis of

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[1] Elford, W. J., Galloway, I. A., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 35.

[2] Theiler, M., and Bauer, J. H., *J. Exp. Med.*, 1934, **60**, 767.

presumably successful multiplication in chick embryo media[3, 4] indicates that insufficient care was taken to rule out contamination with other viruses. Investigations by workers at this Institute and elsewhere are in accord that no propagation of the virus has been demonstrated by methods which have proved successful for the cultivation of most of the other viruses.[5]

The problem of cultivating the virus of poliomyelitis is being pursued not only for the further elucidation of the nature of the virus but also in the hope that successful *in vitro* propagation may facilitate attempts at adaptation to new hosts and tissues and provide new material for further experimentation on active immunization.

A new approach was made by the use of 3- to 4-months-old human embryos, obtained aseptically by Cesarean section. (The authors are indebted to Dr. Lance Monroe, of Bellevue Hospital, for the 2 human embryos used in this investigation.) The brain and cord, the lungs, kidneys, liver, and spleen were stored in the refrigerator, fragments of these tissues being taken for the preparation of media at 3-day intervals. The media were prepared by suspending approximately 100 mg. of one or another of the tissues, thoroughly washed and minced, in 4.5 cc. of Tyrode's solution contained in a 50 cc. Erlenmeyer flask; each flask was inoculated with 0.5 cc. of a Berkefeld N filtrate of a 5% suspension of poliomyelitis cords (M.V. strain) which, by titration, was found to be equivalent to approximately 50 minimal infective doses. One human embryo provided the tissues for the first 3 passages made at 3-day intervals, and a second human embryo became available for further subcultures. Definite multiplication of the virus occurred only in the presence of the nervous tissue. One cc. of the sixth brain and cord culture (containing the original inoculum in a dilution of 1:1,000,000) produced typical poliomyelitis in a *rhesus* monkey, while even the third cultures prepared with other than nervous tissue proved not to be infective. A new set of cultures started with the tissues of the second human embryo again exhibited growth in the nervous tissue medium but not in those containing kidney or lung. That the original embryonic nervous tissue did not contain a virus pathogenic for monkeys was checked by inoculation. The identity of the culture virus with that of poliomyelitis was established by the fact that it gave rise to typical

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[3] Gildemeister, E., *Deut. med. Woch.*, 1933, **59**, 877.

[4] Pauli, P., *Ist. Sieroter. Milanese*, 1934, pp. 1-101.

[5] Personal communications.

flaccid paralysis in monkeys, was transmissible in series by Berkefeld N filtrates, induced characteristic pathological changes in the spinal cord (extensive destruction and neuronophagia of anterior horn cells), was neutralized by human and reinforced monkey poliomyelitis convalescent serum but not by normal monkey serum, led to active immunity against the M.V. virus in recovered monkeys, and finally by a complete lack of pathogenicity for rabbits and mice, which excluded the possibility of contamination with other viruses used in this laboratory. The positive cultures contained no formed bodies which were not found in control, uninoculated flasks.

In view of the apparent special affinity of the virus for nervous tissue even in *in vitro* cultures, similar attempts were made to cultivate the virus in monkey spinal cord tissue and in chick and mouse embryonic brain suspended in Tyrode's solution with or without normal monkey serum, but in not a single instance did even the third passage contain demonstrable virus. When, however, the third culture of the virus in human embryonic brain was transferred to chick embryo brain, it was possible to carry the virus in the latter medium for 3 successive subcultures, but several attempts at further adaptation have so far been unsuccessful. Since additional human embryo tissue was not available, subcultures from chick to human brain medium could not be made.

The following significant points emerge from this investigation: (1) While the method just described is clearly of limited use, it serves to establish that the virus of poliomyelitis can multiply *in vitro*. (2) The propagation of the virus in human embryonic nervous tissue, as contrasted with the complete lack of growth in other tissues derived from the same embryos, emphasizes the limited affinities of the virus of poliomyelitis. (3) Multiplication of the virus can occur in cultures prepared with tissue preserved in the refrigerator for at least a week. (4) In view of the recent discussion of the question of possible infectivity for man of poliomyelitis virus long passaged in monkeys (Flexner[6]), it is interesting to note that the M.V. virus, which has been passed from monkey to monkey for more than 20 years, readily multiplied in human tissue. (5) Although there is as yet no evidence of adaptation to other embryonic nervous tissue, the method of initiating growth in human embryonic brain with subsequent subculture in the embryonic tissue of other species is being pursued further.

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[6] Flexner, S., *Science*, 1935, **82**, 420.

8620 P

Reaction of Rat Mother to Retention of Near Term Dead Fetuses.

Arthur M. Crosman. (Introduced by C. R. Stockard.)

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Corey[1] reported that death of the mother almost invariably took place in cases which involved retention of older (larger) dead rat fetuses. He stated that, "Near-term fetuses which have been either separated from the maternal blood supply in the uterus or placed in the maternal peritoneal cavity bring about the early death of the mother." Death occurred in his cases from 1-3 days after the operation. The present work does not confirm these findings.

Albino rat females were caused to retain varying numbers of near term dead fetuses, both *in utero* and in the body cavity. The uteri were exposed through a medial ventral abdominal incision to allow easy access to both horns of the uterus. The fetuses nearest the Fallopian tube were killed through an incision in the uterine wall, at the periphery of the placenta, just large enough to insert a needle. The end of the needle was bent to form a complete loop in order not to injure the tissue unnecessarily. It was possible to free the entire placenta by rotating such a needle between the placenta and uterine wall. This procedure carefully done does not injure the embryo or embryonic membranes. Abortion of the killed fetuses was prevented by ligation of the uterus. Sterile precautions were always employed.

Two fetuses measuring 19 mm. (c-r) were killed. The remaining young, but one, were not disturbed during the operation. The one was removed as a control. Four days after the operation a litter of 6 healthy young were born. These were nursed by the mother and given excellent care. Apparently the 2 retained dead fetuses produced no disturbance in the general behavior of the mother.

In another case 3 fetuses of 28 mm. (c-r) were killed and left in one horn of the uterus; the other uterine horn not being disturbed. Two days after the operation this female had a litter of 4 living young. These were all eaten by the mother in the next 2 or 3 days. Failing to eat regularly thereafter, the mother was forcibly fed for about 10 days, starting on the 9th day after the operation. At the end of this time she began to eat more regularly although not as well as a normal rat. She thrived fairly well under the same general treatment received by the rest of the rat colony. On the 35th day after the operation a reddish eminence on her side was noticed defining an area where the fetal material had caused an ulceration in the abdominal wall. The animal was sacrificed the next day. Autopsy revealed partially macerated fetuses impacted against the body wall in the region located externally by the presence of the discolored evagination.

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[1] Corey, E. L., *Anat. Rec.*, 1933, **56**, 196.

In still another experiment 8 dead fetuses measuring 30-31 mm. were retained *in utero*. The female failed to eat well so forced feeding was started on the 6th day after operation. This was continued until the mother died 17 days after operation. Nothing particularly abnormal was noted in her general reaction except for anorexia. Her general condition seemed to improve from the 13th to the 16th day and consequently death on the 17th day after operation was unexpected. Autopsy revealed the bulk of the dead fetuses to be so great as to extend up under the liver, nearly to the diaphragm. The intestines were considerably disarranged, and gangrenous in one area with extensive adhesions. It is believed that death of this female was caused by intestinal obstruction with a resultant gangrene. Examination of the retained material showed considerable maceration to have occurred, not only of the fetuses but of the membranes and placentæ as well.

There appears to be some evidence in the literature that necrosing dead fetuses produce greater toxic effect on the mother if retained in the peritoneal cavity rather than *in utero*. Corey[1] reports that dead fetuses *in utero* produce practically no change in the maternal total leucocyte number but if retained in the body cavity a definite leucocytosis results. He further states, "The uterus may thus be said to act in a protective manner against the necrosing fetal tissue in its lumen." From this one might assume that if unenveloped fetuses were placed in the peritoneal cavity the maternal reaction to the presence of these dead fetuses would be greater than when left *in utero*, and therefore, death of the mother resulting from retention of such near term dead fetuses should be more rapid.

As a test of the above statements 2 fetuses measuring 30-32 mm. (c-r) were placed in the peritoneal cavity with placenta and membranes. The embryonic membranes were almost completely removed from the fetuses. Litter mates were removed at time of operation. A rapid and complete recovery from the operation took place and the female appeared normal. At the end of 28 days she was sacrificed and autopsied. The body outline of these fetuses showed less distortion and apparently less maceration had occurred than in comparable cases of retention *in utero*. These fetuses felt quite firm in contrast to the soft yielding fetal mass generally found when fetuses of this size were retained *in utero*. The placentæ were firm and appeared quite shrunken.

In another experiment of the same nature 6 fetuses measuring 32 mm. were placed in the body cavity. The embryonic membranes were torn and folded off these fetuses but in each case were placed in the body cavity with the placentæ. This female seemed healthy and normal; eating well and appearing quite lively. The retained dead fetuses had no apparent effect upon the mother. She was sacrificed and autopsied after 21 days. The appearance of the fetuses and their placentæ was similar to that of those just described.

It appears from the above that retention of near term dead fetuses *in utero* or in the peritoneal cavity does not necessarily result in death of the mother.

8621 P

Gasometric Determination of Carboxyl Groups in Amino Acids.

DONALD D. VAN SLYKE AND ROBERT T. DILLON.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

Ruhemann[1] and Grassmann and von Arnim[2] have shown that the color-forming reaction of ninhydrin with amino acids occurs with evolution of CO_2. We have found that at pH 5, or somewhat lower, in water solution the CO_2 from carboxyl groups adjacent to alpha-amino groups can be quantitatively split off in 3 minutes. The apparatus used is that employed by Van Slyke, Page and Kirk[3] for manometric determination of organic carbon. In the combustion tube of this apparatus are placed a few milligrams of amino acid, 100 mg. of KH_2PO_4, and 50 mg. of ninhydrin made to a volume of 1.0 cc. with water. The tube is connected to the manometric chamber, arranged as for combustions,[3] and the solution is boiled gently for 3 minutes. The evolved CO_2 is measured as in carbon combustions.[3] The reaction appears to be specific for carboxyl groups of amino acids. Other organic acids, such as acetic, lactic, citric, yield no CO_2. Each of the naturally occurring amino acids including proline yields 1 mole of CO_2, except the dicarboxylic acids, aspartic and glutamic. Aspartic yields at once 2 moles of CO_2, while glutamic yields one mole at once, and a second much more slowly (only about 2% in 3 minutes). Peptides do not react, even with the free carboxyl, which is inactivated by the binding of the alpha NH_2 group in peptide linking. Urea does not react.

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[1] Ruhemann, S., *J. Chem. Soc.*, 1911, **99**, 792, 1486.

[2] Grassmann, W., and von Arnim, K., *Annalen der Chem.*, 1934, **510**, 288.

[3] Van Slyke, D. D., Page, I. H., and Kirk, E., *J. Biol. Chem.*, 1933, **102**, 635.

Because other primary amines, urea and ammonia do not interfere, the determination of amino acid carboxyl CO_2 may be more specific for amino acids in biological material than is the nitrous acid reaction. Because of the non-reactivity of the free carboxyl group in peptides the method appears to provide a sharp distinction between peptides and free amino acids.

8622 C

Extraction of Gonadotropic Factors from the Blood. Improved Technic.

ROBERT T. FRANK AND U. J. SALMON.*

From the Laboratories of The Mount Sinai Hospital, New York City.

An improved method for gonadotropic hormone (G.H.) extraction from blood was reported from this laboratory in 1935.[1] By means of this method, both follicle stimulating as well as luteinizing effects were obtained with 40 cc. of blood taken from normal menstruating women, from those past the physiological menopause as well as from surgical castrates.[2] This method, which involved desiccation of the blood with sodium sulphate, was used because it enabled both G.H. and E.S. (estrogenic substance) determinations to be performed on the same specimen.

By the present method the deleterious effect of alcohol upon the G.H. factors is avoided, giving a more complete G.H. extraction without impairing the E.S. yield. Moreover, the extracts obtained by this method are non-toxic, in sharp contrast with extracts by the sodium sulphate method which caused the death of many animals because small amounts of sodium sulphate could not be removed.

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* Hiram N. Vineberg Research Fellow.

[1] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1236.

[2] Frank, R. T., Salmon, U. J., and Friedman, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

Forty cc. of freshly drawn blood is run into 150 cc. of cold acetone. A fine precipitate forms which settles to the bottom. The blood-acetone mixture is shaken in a mechanical shaker for 20 minutes, centrifuged and the supernatant acetone poured off. This is repeated twice more with 125 cc. of fresh cold acetone. The acetone fractions are united, evaporated and utilized for estrogenic hormone determinations.

The acetone precipitate is dried under a fan, powdered and then extracted with 100 cc. of water, the mixture being acidified with dilute HCl to pH 4.8 (Brom-cresolgreen). This mixture is stirred by hand for 10 minutes, centrifuged, and the supernatant aqueous extract decanted. This aqueous extract is filtered through a single layer of gauze to remove suspended particles. To the aqueous extract, 400 cc. of cold acetone are added, a fine buff colored precipitate settling out. The mixture is allowed to stand in the refrigerator over night (45°-50°F.). It is then centrifuged and the acetone discarded. The precipitate is extracted with 5 cc. of water, alkalinized with dilute NaOH to pH 8.5 (thymol-blue). The mixture is centrifuged, the supernatant liquid decanted and its pH adjusted to 7 with dilute hydrochloric acid (Brom-thymolblue). This extract is then injected in 5 divided doses over a period of 60 hours, into an immature female rat weighing 24 to 26 gm. The animal is sacrificed at the end of 96 hours and its ovaries studied in serial section.

With this method we have obtained strong follicle stimulating and luteinizing effects with 40 cc. of blood taken from (a) normal women at the 9th to the 13th day of the cycle; (b) women subjected to surgical castration; (c) women who had passed through the physiological menopause; (d) young women affected with secondary amenorrhea.

For the initial precipitation of the blood we have also investigated the use of 95% ethyl alcohol, pure butyl alcohol, alcohol-acetone and ethyl alcohol-butyl, alcohol-acetone mixtures in place of pure acetone.

While both G.H. and E.S. factors can be determined employing these other precipitants in place of acetone, the most uniform results for both factors were obtained with straight acetone.

8623 C

Protective Action of Copper against Infection with *Mycobacterium tuberculosis* (Bovine) in Albino Rats.

DAVID PERLA.

From the Laboratory Division, Montefiore Hospital, New York City.

The protective action of supplements of copper to the diets of rats against infections with *Bartonella muris, Trypanosoma lewisi* and *Trypanosoma equiperdum* has been reported.[1, 2, 3] The present communication deals with the effect of additions of copper salts to the diet on a subsequently induced infection with *Mycobacterium tuberculosis* (bovine strain) in albino rats. It was observed[4] that the adult rat of our stock, though highly resistant to tuberculous infection, could be infected with 0.1 mg. of a virulent bovine strain of tubercle bacilli and revealed gross lesions of tuberculosis in the lungs within a period of 3 to 5 months.

The rats are from the stock raised in our laboratory for many years and maintained under constant environmental and dietary conditions. The diet has been described[2] and was found to contain the equivalent of about 0.025 mg. of elemental copper per 15 gm. of food. Supplements of copper were given in the form of copper sulphate in amounts equivalent to 0.2 mg. of copper per rat per day.*

Fifty-six rats were divided into 4 groups; 12 received daily supplements of copper in the amounts indicated above; 12 received supplements of iron; 12 received supplements of copper and iron. All supplements were commenced 14 days prior to the injection of the tubercle bacilli, and continued during the entire experimental period. Twenty rats were fed on the normal diet. Each of the rats was injected intraäbdominally with 0.1 mg. of bovine tubercle bacilli ("Ravenel" strain). All the animals were killed 5 months after the injection of the bacteria.

From the table it may be seen that of the rats receiving copper-supplements, 6 showed no evidence of tuberculous lesions at autopsy and 6 had a few scattered tubercles in the lungs (indicated in the

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[1] Perla, David, and Marmorston-Gottesman, J., *J. Exp. Med.*, 1932, **56**, 783.

[2] Perla, David, *Am. J. Hyg.*, 1934, **19**, 514.

[3] Perla, David, *J. Exp. Med.*, 1934, **60**, 541.

[4] Perla, D., and Marmorston, J., *Arch. Path.*, 1933, **8**.

* I am indebted to the Myron L. Walker Co., Inc., Mt. Vernon, New York, for the preparations of copper and iron.

table as "slight infections"). Of the rats receiving iron-supplements alone, none escaped infection; 7 showed large conglomerate lesions in the lungs (indicated as "severe infection" in the table) and marked involvement of the retroperitoneal lymph nodes; 3 showed evidence of a slight infection and 2 of a moderately severe involvement of lungs and lymph nodes. Of those rats which were fed both copper and iron supplements, one escaped infection; 5 had only slight tuberculosis and 6 moderately advanced infections. Of the 20 controls, none escaped infection; 3 showed numerous lesions in the lungs and lymph nodes; 9 moderately advanced lesions in the lungs, and 8 slight infection as manifested by an occasional scattered tubercle in the lungs and little or no lymph-node involvement.

TABLE I.

Effect on Infection with *Mycobacterium tuberculosis* (Bovine-"Ravenel") in Adult Albino Rats of Copper and Iron Supplements to an Adequate Diet.

All the rats were killed 5 months after the intraäbdominal injection of 0.1 mg. of tubercle bacilli.

No. Rats	Supplementary feeding*	Type of infection			
		No evidence	Slight	Mod.	Severe
12	Copper†	6	6	0	0
12	Copper and iron‡	1	5	6	0
12	Iron	0	3	2	7
20	Controls	0	8	9	3

*The supplements were commenced 14 days prior to the injection of the tubercle bacilli.

†The copper was given as copper sulphate in amounts equivalent to 0.2 mg. of elemental copper per rat per day.

‡The iron was given as iron ammonium citrate in amounts equivalent to 2 mg. of elemental iron per rat per day.

The natural resistance of the rat to infection with *Mycobacterium tuberculosis* (bovine strain) can be raised with supplements of copper to the diet. This effect is consistent with previous findings on the influence of copper on infections in the rat. The beneficial effect of copper is probably due to its physiological importance as a catalytic agent in oxidative processes in cellular metabolism.

In these experiments iron-supplements had a deleterious effect on the course of the tuberculous infection and the lesions were more extensive than in the control rats. It may be that an excess of iron over a long period of time results in cellular injury due to excessive storage of the metal and may interfere with the mechanism of resistance to tuberculosis.

The iron is found deposited in large amounts in the macrophagic tissue of the spleen, the Kupffer cells of the liver and in large macrophages within the tuberculous lesions of the lungs.

Menkin[5] has shown that following repeated injections of a dilute solution of ferric chloride in rabbits the iron is deposited in large amounts within and about the tuberculous lesions and its presence tends to favor fibrosis and prolong the life of the animal.

Menkin administered 3 injections a week intravenously each of 6 cc. of a 0.25% solution of ferric chloride, during a period of 4 months. This represents a total amount of approximately 220 to 250 mg. of elemental iron for the entire experimental period. Though this is approximately the same quantity as that used in the experiments here reported, it is probable that the differenes in the animals used may account for the difference in the results.†

8624 P

Relation of Cholane Nucleus to the Female Bitterling Test for Male Hormone.*

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From the Department of Physiology and Biochemistry, New York Homeopathic Medical College and Flower Hospital.

After demonstrating that the female bitterling test was not a test for pregnancy,[1] we have stated[2] that the fraction of male urine containing the male hormones is responsible for the ovipositor elongating reaction. A positive reaction was taken as a lengthening of the ovipositor from a quiescent stage to a length equal to that of the anterior edge of the anal fin, *i. e.*, the ovipositor must reach the end of the fin.

Crystalline theelin and theelol were also tested and although they

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[5] Menkin, Valy, *J. Exp. Med.*, 1932, **55**, 101; 1934, **60**, 463.

† The literature[6-10] on the therapeutic use of copper is reviewed in a previous article.[3]

[6] Bevan, cited by Corper, H. L., DeWitt, L., and Wells, H. G., *J. Am. Med. Assn.*, 1913, **60**, 887.

[7] Von Linden, G., *Beitr. Klin. Tuberk.*, 1912, **23**, 201.

[8] Meissen, E., *Beitr. Klin. Tuberk.*, 1912, **23**, 215.

[9] Strauss, A., *Beitr. Klin. Tuberk.*, 1912, **23**, 223.

[10] Corper, H. J., *J. Infect. Dis.*, 1914, **15**, 518.

* We are grateful to the Littauer Foundation for a grant to aid this investigation and to Mr. C. W. Coates, of the New York Aquarium for his cooperation.

[1] Kleiner, I. S., Weisman, A. I., and Barowsky, H., *J.A.M.A.*, 1935, **104**, 1318.

[2] Kleiner, I. S., Weisman, A. I., and Mishkind, D. I., *J.A.M.A.*, in press.

did not give positive reactions, they sometimes caused a slight lengthening of the ovipositor. We therefore felt it important to determine whether any other substance containing the cholane nucleus would produce this reaction in greater or lesser degree.

We have tested ergosterol† and cholesterol emulsions and solutions of sodium taurocholate according to our method.[2] Small doses of all these compounds produced no reactions. These doses were equivalent to, or several times greater than the doses of male hormone which produced pronounced reactions. Larger amounts of cholesterol, however, produced slight effects while the same amounts of sodium taurocholate were fairly active. For example, while approximately 1.7 mg. of the crude male hormone fraction produced a marked positive reaction when added to 4 liters of water containing 2 bitterlings, 40 mg. of cholesterol caused only slight effects. Doses of 8 mg. of sodium taurocholate caused no reaction, while 25 to 50 mg. gave a moderately strong reaction. Thirty animals were used. It is highly improbable that these weak reactions were due to contaminations of our "C.P." preparations with male hormone. This could be excluded only by the use of synthetic products.

Since bile salts occur in urine in appreciable amounts only in cases of obstructive jaundice, it is evident that ordinarily this factor would not interfere with the test. Moreover, it is doubtful whether even in extreme icterus sufficient amounts of bile acids are excreted to cause any reaction.

It appears that the cholane nucleus, *per se,* is probably not the sole causative factor in the production of the female bitterling (ovipositor) reaction.

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† The ergosterol was generously supplied by Mead Johnson and Co.

8625 P

Absorption Through the Nasal Mucosa of Mice.

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From the Laboratories of the Rockefeller Institute for Medical Research, New York.

Following the technique of Clark[1] and Olitsky and Cox[2] in their investigation of absorption through the mucosa of the nose, mice were inoculated intranasally with 0.02 cc. of a mixture of equal parts of 10% potassium ferrocyanide and 10% iron and ammonium citrate. 0.01 cc. of the solution was allowed to drop in small droplets on the outside of each nostril, from which position the mouse gradually breathed it into the nose. At 2, 5, 15, and 30 minutes and 1, 2, 3, 4, 6, and 8 hours the mice were sacrificed by decapitation; the whole head was dropped into 10% formalin containing 5% HCl. After 5 or 6 days in this fixative, to allow for complete decalcification, each head was divided down the middle line and, after clearing and embedding, microscopical preparations were made from each side. They were lightly stained with Mayer's carmine and mounted.

As a result of the examination of preparations from 40 mice, 12 each at 2 and 15 minutes and 2 each at the other times, it is possible to state that in mice at least, the whole process has reached its maximum in animals killed at 2 minutes and from then on the picture is one of decreasing amounts of Prussian blue in any tissue of the area examined. The granules are dispersed through the lymphatics, the blood vessels, and the subarachnoid space. Previous investigators[1, 2] had not examined preparations earlier than one hour.

At 2 minutes the solution is passing through both olfactory and respiratory mucosa. In the former a few granules are seen between the cells but the great majority appear in streams along the receptor fibrils of the olfactory neuron cells and scattered within the cytoplasm of these cells. Absorption is thus chiefly through these sensory cells. In the respiratory mucosa the granules are thickly clustered over the cilia. They may be seen between the tall columnar cells, or again, within the cytoplasm of these cells, but to a lesser degree than in the olfactory mucosa. This appearance of absorption through the cells is very different from that seen in connection with dead mucosal cells which are stained diffusely blue and contain granules in nucleus as well as cytoplasm.

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[1] Clark, W. E. le G., *Great Britain Rep. Pub. Health and Med. Subj.*, Ministry of Health, No. 54, 1929.

[2] Olitsky, P. K., and Cox, H. R., *Science*, 1934, **80**, 566.

Granules appear in the cytoplasm of some of the cells of the serous glands. In the submucosa, granules of Prussian blue lie scattered in the tissue spaces but are gathered for the most part in small, thin-walled, ramifying lymphatics. Capillaries and venules contain a few granules and the sinusoids in the turbinates contain many. Granules can be seen in capillaries or venules accompanying branches of the olfactory nerve, or running in separate foramina through the cribriform plate.

Under the low power of the microscope, branches of the olfactory nerve supplying areas of olfactory mucosa where absorption is most active, namely, the upper and anterior portion of the septum and the turbinates, appear as blue bands. Under oil immersion it is seen that the perineural spaces are filled with granules and that the granules also lie deep in the bundles between the fibres. This appearance is confined to the smaller branches. The main bundles running up to and through the cribriform plate show granules within the perineural sheath or, as the nerve enters the subarachnoid space, adherent to the outside of the nerve. Granules are also to be seen in the perineural sheath, and occasionally between fibres, of the trigeminal nerve but are fewer than in the olfactory branches.

Granules lie within and line the arachnoid network in the subarachnoid space, especially at the anterior angle of the skull. The pia-arachnoid membrane over the olfactory bulbs is heavily sprinkled with distinct little blue granules.

None is to be seen within the brain itself, save very occasionally in small blood vessels. Granules occur in the subdural sinuses, especially the cavernous.

At 5 minutes absorption is less. In the submucosa there are fewer granules between the nerve fibres and within the perineural sheaths of the smaller branches but the lymphatics and small vessels contain even more than at 2 minutes. Within the cranial cavity granules are less frequent in the pia-arachnoid membrane but elsewhere are as plentiful as before. At 15 minutes there are considerably less than at 5. Absorption has almost ceased through the respiratory mucosa but some still occurs through the olfactory cells. Nerves, lymphatics, and blood vessels in the submucosa show greatly reduced numbers of granules. Granules are fewer in the pia-arachnoid membrane, the subarachnoid space, and the sinuses.

From now on there is a steady decrease in the distribution of

Prussian blue granules anywhere in the tissues. At 8 hours masses of blue granules are still to be seen tangled in mucus over the mucosa. Some absorption is still occurring through the olfactory cells and, very occasionally, between the cells. In the submucosa a very few granules can be seen in the tissue spaces or within lymphatics subjacent to areas of mucosa where absorption is still occurring. Along one or two branches of the olfactory nerve, small clumps of granules appear within the perineural sheath and similar clumps appear within the subarachnoid space. A few scattered granules occur in the pia-arachnoid over the olfactory bulb.

Absorption, therefore, occurs chiefly by way of the olfactory sensory cells and appears at its maximum in mice killed at 2 minutes. Passing chiefly along the nerve fibres, it has reached the subarachnoid space and the pia-arachnoid membrane within this time.

8626 C

Comparison of Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. VIII. Mercuric Chloride.

A. J. Salle and A. S. Lazarus.

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The use of mercuric chloride as a disinfectant dates from the time of Robert Koch.[1] It has long been recognized as one of the most powerful disinfectants known. The germicidal power is exceedingly high in aqueous solution but enormously reduced in the presence of organic matter and some inorganic ions. It is precipitated by proteins and other organic compounds, hard water, alkalies, numerous salts, metals and sulfides.

It appears to be definitely established that mercuric chloride owes its germicidal properties to the positive or mercuric ion. This means that the compound is effective only in the dissociated state. Krönig and Paul[2] found that a 1-500 solution of the mercurial salt in water is much less than twice as active as a 1-1,000 dilution. The addition of any substance suppressing ionization causes a decrease in its germicidal efficiency. Sodium or ammonium chloride is sometimes added to increase the stability of the compound, but the germicidal power is greatly reduced due to a decrease in ionization. It is not applicable to the disinfection of sputum, excreta, and the like due to the formation of a coagulum which prevents further penetration.

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[1] Koch, R., Ueber Desinfection, Mittheil, Kaiserl. Gesendheitsant, Vol. 1, quoted from ''A Handbook on Antiseptics,'' by H. D. Dakin, New York, The Macmillan Co., 1917.

[2] Krönig, B., and Paul, Th., *Z. f. Hygiene,* 1897, **25**, 1.

In previous papers of this series[3-9] comparisons were made of the resistance of *Staphylococcus aureus* and embryonic chick heart tissue to phenol, Merthiolate, Metaphen, Mercurochrome, Hexylresorcinol, iodine, iodine tri-chloride and potassium mercuric iodide. Indices of toxicity were determined by dividing the highest dilution of the germicide that killed the tissue by the highest dilution of the chemical preventing growth of the test-organism. Theoretically the smaller the index the more nearly perfect the chemotherapeutic agent.

The methods employed were the same as those given in the first paper of this series. Widely spaced dilutions were first prepared to determine approximately the least concentration of the germicide required to destroy the bacteria in 10 minutes but not in 5 minutes. Usually one such preliminary series was sufficient. Then a series of dilutions covering a narrow range was prepared to determine more accurately the least concentration necessary to kill the bacteria in the specified period of time. In every case the results were checked a second time. If the second series failed to check, the first tests were repeated until checks were obtained. The same procedure was followed to determine the last concentration of the germicide required to kill the living embryonic tissue, except that a period of 48 hours was used instead of 10 minutes.

A *S. aureus* phenol-coefficient was first determined for mercuric chloride by the method of Reddish.[10] Phenol killed *S. aureus* in a dilution of 1-65 in 10 minutes but not in 5 minutes. The highest dilution of mercuric chloride required to kill the test organism

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[3] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

[4] *Ibid.*, 937.

[5] *Ibid.*, 1057.

[6] *Ibid.*, 1119.

[7] *Ibid.*, 1481.

[8] *Ibid.*, **33**, 8.

[9] *Ibid.*, 393.

[10] Reddish, G. F., The Newer Knowledge of Bacteriology and Immunology, E. O. Jordan and I. S. Falk, University of Chicago Press, 1928.

under the same conditions was found to be 1-16,000. Therefore, the *S. aureus* phenol-coefficient was 246.

A considerable literature has been built up on the bactericidal efficiency of mercuric chloride. Since so much of it is contradictory no attempt will be made to review the results in this paper.

Cultures were prepared from chick heart tissue obtained from 9-day-old embryos. The fragments of tissue were embedded in guinea pig plasma in Carrel flasks. The various dilutions of phenol and mercuric chloride were made in dilute chick embryonic fluid. The plasma, after coagulation, was washed with Tyrode solution to remove the uncoagulable constituents, after which were added the various dilutions of germicide in embryonic fluid. Final observations were made at the end of 48 hours.

The results are summarized in Table I.

TABLE I.
Toxicity of Phenol and Mercuric Chloride to Chick Heart Tissue and Bacteria.

	Highest dilution showing no tissue growth = A	Highest dilution showing no growth of *S. aureus* = B	Toxicity index = A/B	*S. aureus*-phenol Coefficient
Phenol	1-840	1-65	12.9	
Mercuric chloride	1-45,000	1-16,000	2.8	246

Lambert[11, 12] employed human tissue from spleen and lymph glands embedded in a mixture of chick plasma and human serum. He found that the explants were killed by a 1-80,000 dilution of mercuric chloride while a concentration of 1-40,000 was required to kill *S. aureus*.

Later Lambert and Meyer[13] employed rabbit's splenic tissue embedded in homologous plasma. They found that splenic cells were more resistant to the action of the disinfectant than bacteria, the concentrations required being 1-2,500 and 1-10,000 respectively. They concluded that some of the classic antiseptics, including mercuric chloride, approached nearer the "ideal" than certain of the newer preparations.

The results given in Table I show that mercuric chloride is almost 3 times more toxic to tissues than to bacteria. However, it rates next to the iodine compounds and considerably superior to the newer organic mercurial preparation so far studied

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[11] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

[12] Lambert, R. A., *J. Am. Med. Assn.*, 1916, **67**, 1300.

[13] Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

when tested by the tissue-culture method. The germicides may now be placed in the following order on the basis of their toxicity indices: iodine 0.09; iodine trichloride 0.40; mercuric chloride 2.8; Hexylresorcinol 3.0; Metaphen 12.7; phenol 12.9; potassium mercuric iodide 13.3; Merthiolate 35.3; Mercurochrome 262.0.

8627 P

Cysteine in Jensen's Sarcoma.

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With the stimulating effect of cysteine and other sulphydryl compounds upon tissue growth in mind, it was felt that dilutions of these compounds injected into rapidly growing neoplastic tissue might serve as a sufficient stimulation to accelerate maturity and subsequent death of cells. To evaluate the effect of sulphydryl radicals upon the growth of tumor-cells *in vivo*, a group of 6 rats was chosen which had been inoculated with Jensen's sarcoma from 10 to 21 days previously. These tumors varied in size from 4 to 9 cm. in diameter and all were in a normal state of growth, none showing necrosis or evidence of regression. Twenty mg. of a freshly prepared solution of cysteine hydrochloride was injected into the center of each of 5 of these tumors at 2-day intervals for 4 doses. The sixth was kept as a control. Forty-eight hours after the first dose, some tenseness and edema was observed with a subsequent and rather rapid necrosis of tissue in the center of the tumor in each case which subsequently progressed to the periphery. In every instance, all of the tumor-tissue died as the necrotic tissue was extruded, after which the skin healed normally over the ulcerated area. Six months have elapsed since the initial injections and there is no sign of tumor-recurrence.

This experiment has been repeated on 4 groups of animals with the same result following injection of sarcomatous tumor-tissue with cysteine hydrochloride. In each group, except the first, 5 rats were injected and 2 animals maintained as controls. In each case, the control-group showed the usual rate of tumor-growth with no evidence of regression and gradual, progressive enlargement of the tumors until the animals died from them, while the injected tumors regressed and disappeared. No reasonable explanation for this regressive action is at hand.

A series of other substances at the same pH as cysteine are being run. Many of them have caused necrosis but none to date has caused complete regression. The complete results will be reported later.

Lumsden[1] and other investigators have found that certain agents known to cause regression of malignant tumors in animals would also confer an immunity after the tumor has regressed. He remarks that a satisfactory mode of treatment of animal tumors must not only serve as a destroyer of the growth but also as an immunizer. To date, surgery and radiation have more or less satisfactorily served as a means of removal but they have little if any power to invoke an immunity. Conversely, auto-vaccination treatment by inoculation is an excellent method of incurring immunity but it is not easy to maintain a concentration of antibodies in the tumor-area sufficient to destroy all the tumor-cells. He has found formalin to be a reagent which stands between antiserum and the physical methods. It is, however, an obviously unsatisfactory chemical with which to work.

We have found that any recession of a tumor, whether induced or spontaneous, is followed by an immunity such as Lumsden describes. There has been so far, no relation to the age or size of the growth; a young tumor, regressing early, confers the same immunity as an old tumor in a practically moribund animal. Excepting physical agents of removal, such as excision and X-ray, the sequence is consistent. It is apparently related in no way to the substance used but seems more to be dependent upon the absorption of tumor-tissue by the host.

While using cysteine recently as a regressive agent in Jensen's sarcoma of rats, it was noted that in animals where the tumors had disappeared after treatment, reinoculation was not possible. To check this apparent induced immunity, a group of 5 rats was taken which had previously been inoculated with Jensen's sarcoma and which had in turn been treated with cysteine hydrochloride successfully. There had been a complete tumor-regression in each case. Approximately 3 weeks after the tumors had disappeared, these animals were all reinoculated with the same strain of Jensen's sarcoma in the usual way. The inoculation was unsuccessful in each case. A similar group of 4 animals was subsequently subjected to the same treatment and with identical results.

Conclusions: (1) Cysteine hydrochloride, when injected directly into Jensen's sarcoma in the white rat, will cause necrosis and tumor-regression. (2) Animals in which tumor-regression has occurred after the use of cysteine hydrochloride are immune to further inoculation of the same strain of rat sarcoma.

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[1] **Lumsden, Thomas, *Am. J. Cancer*, 1931, 15, 563.**

8628 C

Nitrogen Distribution in Human Placental Globulin.*

J. S. DRAGE AND W. M. SANDSTROM.
(Introduced by R. A. Gortner.)

From the Division of Agricultural Biochemistry, University Farm, St. Paul, Minnesota.

It seemed desirable to analyze placental globulin to determine its chemical composition by nitrogen values. Harding and Fort[1] have reported such an analysis of the whole placenta but this contains several proteins as well as other materials which may alter the proportion of nitrogenous fractions as determined by the Van Slyke method.

To prepare the globulin, mature, normal placentas were collected in sterile containers, washed, cut up, and each extracted with

TABLE I.

	Drage and Sandstrom, Placental globulin	Cavett,[3] Serum-pseudo-globulin	Widdowson[7] serum-globulin	Banzhaf, Suguira and Falk[2]			Harding and Fort,[1] whole placenta
				Normal serum-globulin	Anti-tetanic serum-globulin	Anti-diphtheric antiserum globulin	
Humin N	2.64	2.44	0.53	2.0	2.5	2.8	3.17
Ammonia N	8.40	8.85	9.35	6.00	7.4	7.7	6.40
Basic N	26.32	26.93	24.61	28.6	25.5	24.2	35.18
Arginine N	10.23	10.76	10.67	10.8	9.7	9.8	24.00
Cystine N	1.02	0.72		3.0	2.5	3.3	1.43
Histidine N	4.90	2.95	4.21	3.7	2.3	2.0	2.43
Lysine N	10.29	12.95	9.78†	11.1	11.0	9.1	7.31
N in filtrate from bases	61.32	62.87	61.32	64.40	66.5	66.3	51.72
Amino N	60.12	58.53	57.75	61.4	64.4	64.2	48.35
Non-amino N	1.20	4.34	3.57	3.00	2.1	2.1	3.37
P.T.A. Humin N	1.11						
Total recovery (% N)	99.79	101.09	99.33	101.0	101.9	101.0	99.84

† Lysine plus cystine.

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* Published as Journal Series Paper No. 1419, Minnesota Agricultural Experiment Station.

[1] Harding, V., and Fort, C., *J. Biol. Chem.*, 1918, **35**, 29.

[2] Banzhaf, E., Suguira, K., and Falk, K. G., *J. Immunol.*, 1916, **2**, 125.

150 cc. of 2% solution of sodium chloride at 2°C. for 48 hours. The liquid was decanted, centrifuged, and mixed with an equal volume of saturated solution of ammonium sulfate. The "globulin" was recovered by centrifuging and filtering on hardened paper, dissolved in 2% sodium chloride, and dialyzed until free from sulfate and chloride ions. The precipitate was then recovered on hardened paper and oven-dried. From 40 placentas a yield of 10.5 gm. of globulin resulted; it contained 12.73% nitrogen and 0.61% ash.

The nitrogen-distribution was determined by the method of Van Slyke. The values reported are the averages of duplicate determinations calculated as percentages of the total nitrogen. Some values reported in the literature are given for comparison.

The distribution of nitrogen in placental globulin is essentially that found by Cavett[3] and Widdowson[7] in human serum-globulin, and very similar to the values reported by Banzhaf, Suguira and Falk[2] for normal serum-globulin; also to the values for the globulins of normal and colostral milks as reported by Crowther and Raistrick[8] and those reported by Hartley[5] for animal serum-globulins. This comparison is based largely upon the values for ammonia and arginine which are determined directly and with reasonable accuracy. With the exception of cystine, which is present in smaller quantities, the remaining bases also agree closely. The analysis of whole placental tissue, however, presents an entirely different picture due to other constituents.

8629 P

Fate of Parenterally Administered Crystalline Urobilin; Urobilin Tolerance Test of Liver Function.

CECIL JAMES WATSON. (Introduced by H. A. Reimann.)
From the Department of Medicine, University Hospitals, Minneapolis.

Crystalline urobilin, as isolated from feces by the writer's method,[1, 2] may be administered intramuscularly or intravenously without untoward reaction. In the present investigation it was customary to dissolve 50 mg. of the recrystallized hydrochloride in 2 cc. of 95% alcohol. After dilution with 8 cc. of physiological saline containing .1 gm. of sodium bicarbonate the resultant solution was sterilized in the autoclave for 15 minutes at 15 lb. pressure. Recovery experiments have shown that the substance is not damaged by this treatment.

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[3] Private communication, Cavett, J. W.

[5] Hartley, P., *Biochem. J.*, 1914, **8**, 541.

[7] Widdowson, E. M., *Biochem. J.*, 1933, **27**, 1321.

[8] Crowther, C., and Raistrick, H., *Biochem. J.*, 1918, **10**, 473.

[1] Watson, C. J., *J. Biol. Chem.*, 1934, **105**, 469.

[2] Watson, C. J., *Z. physiol. Chem.*, 1935, **233**, 39.

Urobilin does not give an Ehrlich reaction; consequently, it was surprising to find, after parenteral injection of the above mentioned solutions, that the Ehrlich reaction of the urine regularly became positive or intensified within one-half to 2 hours. It was evident that the substance had become reduced somewhere in passing from muscle or blood stream to the urine. The possibility had to be considered that the added load on the liver occasioned by a sudden addition of 50 mg. of urobilin to the circulation had caused it to refuse native urobilinogen coming from the bowel. This possibility was excluded by administration of crystalline urobilin to an individual with complete neoplastic common duct obstruction, whose urine and feces contained no urobilinogen. Following an injection of 50 mg., either intramuscularly or intravenously, the Ehrlich reaction of the urine became strongly positive within one to 2 hours. Studies are now in progress to determine the site of this reduction in the body. It is evident that all of the injected substance is not reduced, since unchanged urobilin may be demonstrated spectroscopically in the fresh urines obtained one-half to 4 hours after its administration.

In the normal individual, following intramuscular injection of 50 mg. of crystalline urobilin, the fraction appearing in the urine is relatively small. Both in the normal and in individuals with liver disease, except where renal insufficiency is also present, all or nearly all of this fraction appears within 6 to 8 hours after injection. However, it is more reliable to compare the 12 or 24-hour urobilinogen excretion preceding injection with that for a similar period immediately thereafter. As yet studies have been limited to individuals whose 24-hour urine urobilinogen was normal (0-4 mg.) or not more than slightly elevated. The method used depends upon the Ehrlich reaction and is described elsewhere.[3] The data in Table I suggest that the amount excreted in the urine following injection of 50 mg. of urobilin, may quite possibly be correlated with the functional state of the liver.

Because of normal variation in urobilinogen content of the

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[3] Watson, C. J., *Am. J. Clin. Path.*, in press.

TABLE I.

Diagnosis	How given	Urine Urobilinogen Before injection	Urine Urobilinogen After injection	Period of Collection hr.
1. Normal	a. Intramuscularly	Trace	2.01	24
	b. Intravenously	”	5.04	24
2. Normal (previous catarrhal jaundice; mild alcoholism)	Intramuscularly	2.6	4.6	24
3. Normal	”	Trace	2.1	24
4. Normal (quiescent gastric ulcer)	”	0	2.6	12
5. Probable cirrhosis of the liver. (Previous jaundice; enlarged spleen, bleeding varices.)	a. ”	2.4	11.5	12
	b. Intravenously	2.6	32.0	12
6. Complete common duct obstruction due to metastatic carcinoma; (hard, nodular liver).	a. Intramuscularly	0	12.3	24
	b. Intravenously	0	22.3	24
7. Hepar lobatum (ascites, no jaundice)	Intramuscularly	5.3	10.1	24
8. Recent neo-arsphenamine icterus; cystotomy, pyelonephritis, renal insufficiency.*	a. Intramuscularly	5.1	10.5	24
	b. Intravenously	5.5	11.2	24

* In this instance excretion of the injected urobilin was obviously slowed since the qualitative tests did not become positive shortly after injection as is usually true.

feces,[4] a single injection of 50 mg. of urobilin would be insufficient to determine the fraction lost in the feces. The following experiment indicates that this fraction is relatively small.

460 mg. of crystalline urobilin were given intramuscularly, in divided doses not exceeding 50 mg. each, during the first 6 days of an 8-day period in which the total amount of urobilinogen in the feces was 400 mg. Based on estimations made during periods of similar length before and afterwards, the expected excretion, apart from the administered urobilin, was 268 mg.† The difference of 132 mg., or 29% of the injected amount, may be considered to represent the fraction reappearing in the feces. Since the amounts in the urine were negligible, it seems evident that the majority of the injected substance was retained.

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[4] Watson, C. J., *Arch. Int. Med.*, in press.

† The subject of this study suffered from a mild idiopathic hypochromic anemia; a disease characterized by reduced urobilinogen excretion.[4]

8630 P

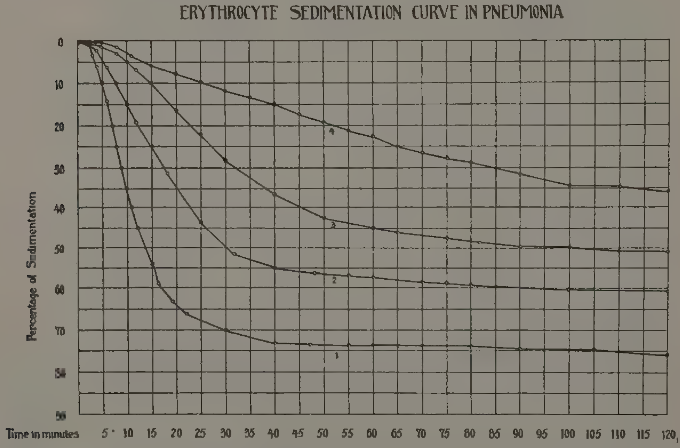
Blood Sedimentation Curves in Pneumonias.

Clyde Brooks.

From the Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans.

A new micro-pipette and a new method for measuring the sedimentation rate of red blood corpuscles has been described.[1] Using this new pipette and method, the present study was made on the blood sedimentation rate in severe cases of pneumonia, both of the broncho and lobar types.

Measurements of the sedimentation rates were made at frequent intervals throughout the course of the disease and for several weeks after what is ordinarily regarded as complete recovery.



The main results are summarized in Fig. 1. In this figure, Curve 1 shows a very rapid sedimentation rate with the corpuscles reaching a low level when they cease settling. This kind of curve is typical for the early stages of severe pneumonias where the patient is critically ill with high fever, and consolidation either beginning or well under way. The outlook in this stage is critical.

Curve 2 is typical of a later stage when the patient has markedly improved, showing little or no fever, and with other clinical signs also much improved. Resolution may be well under way or nearly completed when the sedimentation curve is like that of number 2. The prognosis here is good.

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[1] Brooks, Clyde, *J. Lab. and Clin. Med.*, in press.

Curve 3 is typical of cases which are in the later stages of resolution. The patient is convalescent. During the rapid absorption of material from the lungs in resolution the sedimentation rate remains quite rapid. Not until the lungs are almost clear does the sedimentation rate improve to the extent shown in Curve 3. Most patients leave the hospital before the sedimentation curve shows as much improvement as that in Curve 3.

Curve 4 shows a typical normal curve which is not seen in cases of severe pneumonia until several weeks after the patient has been discharged and pronounced sound and well as judged by the physical findings and the X-ray. This indicates that the sedimentation test is more sensitive to lung pathology than the X-ray or the physical signs.

In cases of severe pneumonia where the patient does not improve but proceeds to grow worse, the sedimentation curve is more rapid and falls to a lower level than that of Curve 1. So, in curves which are more rapid and fall lower than Curve 1, the outlook is bad.

8631 C

Effects of Thorium, Zirconium, Titanium and Cerium on Enzyme Action.

BERNARD S. GOULD. (Introduced by J. W. Williams.)

*From the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge.**

Roussy, Oberling and Guerin[1] have reported that thorium dioxide is carcinogenic when administered intraperitoneally to rats. Doerr[2] and Hara[3] report that thorium and some of the associated periodic members agglutinate bacteria, red blood cells, spores, and precipitate proteins. The effects produced upon enzymes by carcinogenic substances, especially arsenites and hydrocarbons, have been rather extensively investigated. Recently Sure *et al.*[4] found marked reduction in the blood serum amylase, blood serum esterase and in the trypsin of tumour bearing albino rats. Pancreatic amylase and blood serum phosphatase were only slightly decreased according to their findings. The present communication reports the results of a series of experiments upon the effects of thorium, zirconium and titanium which are members of Group IV-A of the Periodic System, and cerium, which is a Rare Earth metal but which shows many physiological properties in common with these others, upon amylase, invertase, phosphatase and trypsin activity. The salts employed were thorium nitrate, $Th(NO_3)_4$; cerium nitrate, $Ce(NO_3)_3 \cdot 6\ H_2O$; zirconium nitrate, $Zr(NO_3)_4$; and titanous chloride, $TiCl_3$, which was allowed to oxidize and must be considered as tetravalent titanium.

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* Contribution No. 70 from the Dept. of Biology and Public Health, Mass. Inst. of Technology, Cambridge.

[1] Roussy, G., Oberling, C., and Guerin, M., *Bull. Acad. Med.*, 1934, **112**, 809.

[2] Doerr, R., *Kolloid Z.*, 1920, **27**, 277.

[3] Hara, S., *Arch. Exp. Path. Pharm.*, 1923, **100**, 217.

[4] Sure, B., Kik, M. C., Buchanan, K. S., Thatcher, H. S., and DeGroat, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 658.

Amylase activity was determined by estimating the amount of maltose liberated from a one per cent starch solution by a purified bacterial amylase *(B. mesentericus)* (Kindly supplied by the Wallerstein Laboratories, N. Y.) by the iodine titration method of Willstatter-Schudel.[5] A veronal buffer[6] at pH 6.8 was employed. Inorganic phosphate buffers were avoided since the phosphates of the rare metals are very insoluble. Twenty-five cc. of one per cent starch solution, 10 cc. veronal buffer, 2.0 cc. of 10^{-2} M rare metal salt were brought to 60°C. and 1.0 cc. of enzyme solution containing 2.0×10^{-2} mg. solid per cc. was added. The final rare metal salt concentration was 5.26×10^{-4} M. Incubation was at 60°C. The results of an experiment are given in Table I. It can be seen that only thorium exerts any appreciable inhibition of amylolytic activity. From a preliminary experiment without buffer all of the metal salts completely inhibited enzyme activity due to the marked acidity of the salts. Cerium, however, showed slight activation over the control, approximately 30%, as determined both by the time to reach the achromic point and by the amount of maltose liberated from the substrate. The thorium-inactivated amylase could be re-activated by the addition of an excess of 25% KH_2PO_4-

TABLE I.
Influence of Salts of Rare Metals on the Hydrolysis of 1% Starch by Purified Amylase (*B. mesentericus*) at 60° C. Rare Metal Concentration = 5.26×10^{-4} M.

Time (min.)	10	20	30	45	60
		% Inhibition			
Thorium	47.7	39.3	31.5	33.5	45.4
Zirconium	3.8	10.5	6.3	4.4	9.0
Titanium	9.2	14.6	11.6	16.5	23.4

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[5] Willstatter, R., *Unters. uber Enzyme*, Berlin, 1928, 1, 2.

[6] Michaelis, L., *J. Biol. Chem.*, 1930, **87**, 33.

K_2HPO_4 (1:1) which precipitates the metal as the insoluble phosphate. The precipitate is filtered off and the supernatant shows complete re-activation. Similar results were obtained with *taka-diastase* (1:200).

Invertase or saccharase activity was determined both by measuring the amount of reducing sugar produced in the hydrolysis of a sucrose-buffer solution by the method of Sumner and Howell,[7] and by determining the rate of hydrolysis, polarimetrically, of a standard buffered sucrose solution by a purified yeast invertase preparation (Wallerstein). The substrate was 6.5% sucrose-acetate buffer solution at pH 4.5. The enzyme preparation employed in the Sumner-Howell reducing sugar method was a washed suspension of yeast cells (0.5 gm. per cc. in 0.85 NaCl). Five cc. of the substrate, 1.0 cc. of 5.0×10^{-3} M rare metal salt solution and 1.0 cc. of cell suspension were incubated at 25°C. and, periodically, analyses were made for reducing sugar by the dinitro-salicylic acid method of Howell.[8] The results are shown in Table II.

Using 1.0 cc. of 1.0% purified invertase solution in place of the cell suspension the rate of hydrolysis was determined polarimetrically at 25° and the calculated reaction constants are recorded in Table II. The magnitude of the effects produced by the salts of these rare metals can be conveniently estimated by a comparison of the relative rates of hydrolysis as indicated in the table. Again we find that cerium accelerates the enzyme action while thorium, zirconium and titanium produce slight inhibition with titanium showing the most marked effect.

The results of the experiments indicate that action is not due to the agglutination of the cells by the rare metals since the same results were obtained with the soluble enzyme as were obtained with the cell suspension. Moreover, cerium accelerates activity in spite of the fact that it agglutinates the cells.

The effects of the rare metal salts on phosphatase activity were determined by the method described by Bodansky[9] and the inorganic phosphate liberated was determined by the method described by Fiske and Subbarrow.[10] One cc. of fresh, clear horse blood serum was added to 9.0 cc. of buffer-phosphate ester (2.5 gm. sodium-β-glycerophosphate (E. K. Co.) and 2.12 gm. sodium barbital in 450 cc. distilled water) and 1.0 cc. of rare metal salt to give a

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[7] Sumner, J. B., and Howell, S. F., *J. Biol. Chem.*, 1935, **108**, 51.

[8] Sumner, J. B., *J. Biol. Chem*, 1925, **65**, 393.

[9] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

[10] Fiske, C. H., and Subbarrow, Y., *J. Biol. Chem.*, 1925, **66**, 387.

TABLE II.

Influence of Salts of Certain Rare Metals on Production of Reducing Sugars from Sucrose by Yeast Invertase at 25°. Rare Metal Concentration $= 7.14 \times 10^{-4}$ M.

Time (min.)	Control	% of reducing sugar produced compared to control Thorium	Titanium	Zirconium	Cerium
5	100	95.3	92.2	102.6	108.1
10	100	102.6	62.5	80.0	121.0
20	100	94.3	61.4	84.4	122.6
30	100	91.3	41.7	85.8	111.1

Effect of Salts of Certain Rare Metals on the Reaction Constant.

Time of hydrolysis (min.)	$K_{control}$ (calculated)	K_{Th}	K_{Ti} (calculated)	K_{Zr}	K_{Ce}
5	.0413	.0393	.0224	.0352	.0435
10	.0333	.0328	.0275	.0314	.0357
15	.0320	.0293	.0270	.0293	.0346
20	.0265	.0244	.0229	.0250	.0295

The reaction constant, K, was calculated with the use of the equation $K = 1/t \log (a/a - x)$; time, t, is expressed in minutes; for a is used the difference between the observed rotation at 0 time and that of an equilibrium mixture of the hydrolytic products of the sucrose solution; $(a - x)$ is taken as the observed rotation at time t, minus the rotation of the equilibrium mixture: $K = 1/t \log (a_{0} - a_{\infty}) - \log (a_{t} - a_{\infty})$

Invertase concentration was 0.143%; the final salt concentration was 0.000714 M. Temperature of hydrolysis, 25° C.

final concentration of rare metal salt of 2.73×10^{-3} M, 1.83×10^{-3} M, 9.1×10^{-4} M, 4.6×10^{-4} M and 2.3×10^{-4} M at 37°C.

Incubation was continued at 37° for 1.0 and for 24 hours. The results tabulated in Table III are expressed as the per cent inhibition as measured against a control in which distilled water replaced the rare metal salt. From the results it is apparent that titanium and zirconium inhibit blood phosphatase very markedly even in very low concentrations. Thorium shows slight inhibition but cerium inhibits only in very high concentrations.

The effects of the rare metal salts on trypsin activity were determined using the method of Willstatter *et al.*[11] Casein solution

TABLE III.

Per cent Inhibition Produced by Various Concentrations of Salts of Certain Rare Metals on Blood Phosphatase after 1 hr. and 24 hr. Incubation at 37° C.

Salt conc.	Thorium 1 hr.	 24 hr.	Cerium 1 hr.	 24 hr.	Zirconium 1 hr.	 24 hr.	Titanium 1 hr.	 24 hr.
0.00273 M	39.4	28.6	28.6	0	80.6	59.0	90*	86.0
0.00182 M	33.3	25.0	13.0	0	65.0	50.0	90*	81.0
0.00091 M	25.0	22.3	13.0	0	51.1	46.8	66.1	49.2
0.00046 M	23.0	15.3	0	0	48.9	22.0	53.1	48.0
0.00023 M	9.0	11.8	0	0	31.0	20.0	42.0	44.3

*More than 90% inhibition.

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[11] Willstatter, R., *Z. physiol. Chem.*, 1926, **161**, 191.

(6.5%) was digested with commercial trypsin at pH 8.9 in the presence of the rare metal salts and in an untreated control. Even in concentrations as high as 1.1 x 10^{-3} M no effects could be observed.

Experiments with purified lipase and esterase which will be reported in a subsequent communication indicate that these enzymes are not appreciably affected by the salts studied.

It is questionable whether these results obtained *in vitro* conditions represent the effects that would be observed *in vivo*. The results observed by Sure *et al.*[4] are not paralleled completely since we have found that trypsin, lipase and esterase are unaffected or only slightly affected by these metals, one of which has been reported as carcinogenic. A more justifiable comparison must await the results of the effects of these metals on *in vivo* enzymic activity.

8632 C

Sex-Difference in Susceptibility to Dinitrophenol Intoxication in Anesthetized Cats.*

P. E. Chamberlin and V. E. Hall.

From the Department of Physiology, Stanford University, California.

In the course of an investigation concerning the interaction of the calorigenic actions of 2-4 dinitrophenol and epinephrine, we have observed a marked sex difference in the susceptibility to dinitrophenol intoxication, as judged by the survival time to a dose which was, under our experimental conditions, almost invariably fatal. There do not appear to have been any reports in the literature concerning sex-differences in the action of dinitrophenol.

Adult cats, previously starved for 24 hours, were anesthetized with pentobarbital (37.5 mg. per kilo intraperitoneally), and attached to a closed circuit metabolism apparatus by means of a tracheal cannula. After a preliminary control period of one-half hour, a quantity of sodium 2-4 dinitrophenolate adequate to yield 10 mg. per kilo of dinitrophenol was injected intramuscularly. In certain experiments, an epinephrine solution was infused intravenously at a rate of 0.001 mg. per kilo per minute during the period of dinitrophenol action. (The Ringer's solution used contained 1 part of defibrinated blood to 10 parts of saline to protect the epinephrine against oxidation.[1]) In the remaining experiments Ringer's solution was infused at the same rate. Oxygen consumption, rectal temperature, and respiratory rate were followed.

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* Supported in part by the Rockefeller Fluid Research Grant of the Stanford University School of Medicine.

TABLE I.

Survival Time of Cats Receiving Dinitrophenol Alone, and Dinitrophenol Plus Epinephrine.

	Males		Females	
	DNP alone	DNP plus Epinephrine	DNP alone	DNP plus Epinephrine
	283	140	65	16
	155	115	140	45
	280	107	76	10
	245	53	45	60
	168	169	25	46
			55	27
			19	
			60	
Av.	226	117	61	34

In the table, the survival times of the animals are given in minutes after the injection of dinitrophenol. Among the cats receiving dinitrophenol alone, the males survived on the average 226 minutes, the females 61 minutes. The reliability of this difference was calculated by the variance method of Fisher.[2] The odds that this difference could have appeared by chance were less than 1 in 5000. Thus, even though the number of cases is small, the reliability of the difference is established beyond reasonable doubt.

Among the cats given epinephrine as well as dinitrophenol, a similar difference appeared, the male survival time averaging 117 minutes, the female 34 minutes. The odds that this difference could have appeared by chance were 8 in 1000, which establishes the difference as reliable.

Within each sex, the addition of epinephrine to the dinitrophenol decreased the survival time by about one-half. The odds that this difference could have occurred by chance were for the males less than 1 in 90. For the females the chances were only 1 in 5 on account of their greater variability.

Lest the differences reported above should be due, not to the sexual factor, but to some factor by chance associated with sex in this group of animals, we attempted to correlate survival time with body weight, rectal temperature at the time of injection, initial control metabolic rate and dose of pentobarbital required to maintain anesthesia. In none of these cases was any significant correlation found.

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[1] Wiltshire, *J. Physiol.*, 1931, **72**, 88.

[2] Fisher, R. A., Statistical Methods for Research Workers, third edition, London, 1930, p. 107.

However, it was noted that the males showed a greater metabolic rate (per unit surface area) at the moment when the first signs of the fatal collapse (slowing of breathing, decreased oxygen consumption[3]) appeared. Pooling all 4 groups of animals, we find a positive correlation of 0.45 between this "critical" metabolic rate and the logarithm of the survival time, a correlation which might have arisen by chance in this population in 1 case in 20 (Fisher[2]). The existence of this correlation suggests that the sex difference in survival time may be due to the males having a greater capacity to deliver oxygen to the tissues than the females, death resulting when a certain oxygen debt has been incurred. However, the correlation is equally well explained on the assumption that death, occurring as a result of some action of dinitrophenol, relatively early in the course of the rise in metabolism would curtail the time available for the rise and consequently its magnitude.

In seeking a physiological sex difference to which the difference in susceptibility to poisoning with dinitrophenol, a drug which involves particularly actively the metabolism of fats,[3] might be referred, we were reminded of the work of Deuel and associates,[4] who have found that female rats, guinea-pigs and human beings manifest ketosis more readily than do the males. A second case in which shortened survival time after dinitrophenol may well have been accompanied by ketosis occurred in the experiments of Tainter, Boyes and DeEds,[5] who observed that depancreatized dogs succumb more rapidly to the drug than do control operated animals. A third case is constituted by the observations by Baudouin, Bénard, Levin and Sallet,[6] among others, that epinephrine, in doses comparable to those which we found to shorten survival time after dinitrophenol administration, caused a marked ketonuria.

The above considerations have suggested to us the possibility that the metabolic disturbance of which ketosis is the presenting manifestation is a factor in predisposing animals to dinitrophenol intoxication. This and other possible explanations of the sex difference reported herein are now being investigated in this laboratory.

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[3] Hall, Field, Sahyun, Cutting, and Tainter, *Am. J. Physiol.*, 1933, **106**, 432.

[4] Deuel and Gulick, *J. Biol. Chem.*, 1932, **96**, 25; Butts and Deuel, *J. Biol. Chem.*, 1933, **100**, 415.

[5] Tainter, Boyes, and DeEds, *Arch. internat. Pharmacodyn. et Thérap.*, 1933, **45**, 235.

[6] Baudouin, Bénard, Levin, and Sallet, *C. r. S. B.*, 1935, **120**, 860.

8633 P

Comparison of Capacity of Some Nitrated Phenols to Stimulate Respiration of Yeast.*

J. FIELD 2ND., A. W. MARTIN AND S. M. FIELD.
From the Department of Physiology, Stanford University.

We have shown that the action of several of the nitrated phenols on the respiration of yeast may be excitatory or inhibitory, depending on the dosage, and that the undissociated form is the active agent in such stimulation or inhibition.[1, 2, 3] A method for comparing the activities of nitrated phenols as metabolic stimulants has been developed and applied to 3 of the dinitrophenols and to the mononitrophenols.[3] We present herewith the results of experiments designed to compare the activities of 5 nitrated phenols which have been used extensively as metabolic stimulants. These are 2-4 dinitrophenol,[4] 4-6 dinitro-o-cresol,[5] 2-4 dinitro-α-naphthol,[6] 2-4 dinitro-cyclo-pentyl phenol[6] and 2-4 dinitro-cyclo-hexyl phenol.[6] The last 2 compounds were provided through the kindness of Dr. M. L. Tainter, who obtained them from Hoffman, LaRoche and Company. In every case aqueous solutions of the sodium salts were employed.

The pure yeast culture and the experimental methods have been described elsewhere.[2, 3] In every case the yeast, after preliminary washing, was suspended in 1% glucose made up in 0.1M phos-

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* Supported in part by Grant 358 from the Committee on Scientific Research of the American Medical Association.

[1] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[2] Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

[3] Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Pharm. and Exp. Therap.*, 1935, **53**, 314.

[4] Magne, H., Mayer, A., and Plantefol, L., *Ann. physiol. physicochim. biol.*, 1931, **7**, 269.

[5] Dodds, E. C., and Pope, W. J., *Lancet*, 1933, **2**, 352.

[6] Heymans, C., and Casier, H., *Arch. internat. de Pharmacodynamie et de Thér.*, 1935, **50**, 20.

TABLE I.
Effect of Optimal Concentrations of 5 Nitrated Phenols on Respiratory Rate of Yeast.

	Optimal concentration at pH 6.8			Aver. respiratory rate†	Respiratory rate Control = 100
Compound	Total Mg. %	Total Molar	Free acid Molar		
None	—	—	—	35.3	100
2-4 dinitrophenol	40*	1.79×10^{-3}	2.96×10^{-6}	51.7	146.4
4-6 dinitro-o-cresol	10	5.05×10^{-4}	7.96×10^{-6}	52.5	148.8
2-4 dinitro-α-naphthol	10	4.27×10^{-4}	—	38.4	109.8
2-4 dinitro-cyclo-hexyl-phenol	1.75	6.58×10^{-5}	—	52.5	148.8
2-4 dinitro-cyclo-pentyl-phenol	1.25*	4.56×10^{-5}	—	51.9	147.0

*Weighed as the sodium salt.

†Aver. respiratory rate is given in cu. mm. of oxygen at N.P.T. per 10^8 cells per hour.

phate buffer, pH 6.8, and the respiratory rate was measured in the conventional form of Warburg manometer in a water bath at 25 ± 0.02°C. Each series of comparisons involving the 5 compounds was performed on yeast from a single Orla-Jensen plate, incubated for 96 hours at 25°C.

The concentrations of the nitrated phenols which evoked maximal stimulation of yeast respiration in glucose-phosphate (optimal concentrations) were determined in a series of 6 to 12 preliminary experiments on each compound. These concentrations are given in Table I, together with average values for the control and experimental respiratory rates in 2 series of comparisons.

It is shown in Table I that optimal concentrations of all the agents tested, except dinitro-α-naphthol, evoke substantially the same degree of metabolic stimulation. This finding suggests that, for our yeast culture and under the conditions of these experiments, there is an upper limit or "respiratory ceiling," beyond which the nitrated phenols cannot raise the respiratory rate. It is interesting that these are the nitrated phenols which other observers have reported to be among the most active metabolic stimulants.[4, 5, 6]

A more detailed comparison is possible for the compounds of known dissociation constant *viz.* dinitrophenol and dinitrocresol. Both, in optimal dosage, evoke the same degree of metabolic stimulation, but the optimal dosages are quite different, as shown in the table. It is to be noted that while the total optimal concentration of dinitrocresol is lower than that of dinitrophenol, the reverse relation obtains when the free acid concentrations are compared, and there is no doubt that for yeast this second comparison is the more fundamental.[1, 2, 3]

8634 P

Prolonged Injection of a Thyrotropic Extract Without Development of Refractoriness.*

Sidney C. Werner. (Introduced by P. E. Smith.)

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It has been uniformly reported that repeated injections of hypophyseal extracts containing the thyrotropic hormone fail to stimulate the thyroid gland beyond 4 or 5 weeks. The BMR and thyroid structure return to normal. This has been explained by Collip[1] as due to the increase of a normally present "antihormone." This theory has also been applied to the other hypophyseal hormones.

Results obtained in this laboratory with 2 different hypophyseal preparations containing the thyrotropic hormone do not support the "antihormone" hypothesis. The development of refractoriness has been found to depend on the type of extract in which the hormone is administered. The thyrotropic effect has been determined by the BMR, the microscopic structure and total iodine content of the thyroid gland. The author is very grateful to Dr. G. L. Foster of the Department of Biochemistry for his kindness in doing the iodine determinations. The determinations were made by his modification of Leipert's 1934 method.

Male guinea pigs, 250-350 gm. in weight, were used. They were maintained under constant conditions in a room in which no iodine was kept or used. The 2 preparations employed were made from beef anterior hypophyses by the van Dyke and Wallen-Lawrence Na_2SO_4 method[2] and by the flavianic acid method of Meyer.† The BMR was measured after a 13-16 hr. period of fasting in a closed system apparatus maintained at 30°C. ± 0.1°.

A. *Treatment with the Na_2SO_4 preparation.* Eleven animals

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* Aided by a grant, administered by Dr. P. E. Smith, from the Committee for Research on Problems of Sex, the National Research Council.

[1] Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, **1**, 28.

[2] van Dyke, H. B. and Wallen-Lawrence, Z., *J. Pharm. and Exp. Therap.*, 1930, **40**, 413.

† A modification, devised by Dr. Meyer, of the original method published by Evans, Meyer and Simpson (Mem. U. Calif., vol. 11, 1933) was used in making the flavianate extract.

The Na_2SO_4 preparation used was "Phyone" made by Wilson and Co. and provided through the courtesy of Dr. David Klein.

were injected daily subcutaneously with one cc. of the Na_2SO_4 preparation for 23-40 days. All of these showed a pronounced increase in the BMR during the first 2 weeks of treatment. Ten of these animals showed a return of the BMR to normal. Three of the refractory animals were autopsied and had structurally normal thyroids. The eleventh animal had a slightly elevated BMR on the 25th day of treatment but a normal gland at autopsy 3 days later. The findings from this group thus confirm the results obtained by Collip,[1] Friedgood[3] and others. No iodine determinations were made.

B. *Treatment with the flavianate preparation.* Sixty-nine guinea pigs were injected subcutaneously for 1-88 days with the flavianate preparation, 0.2-0.4 gm. equivalent of dried beef anterior hypophysis being given daily. This dosage is roughly equal in potency to one cc. of the Na_2SO_4 preparation. Forty-three of the 69 animals were autopsied by the 38th day. Thirty-nine of these gave no evidence of refractoriness. Their thyroids showed intense activation structurally and their total iodine was at the low level characteristic of the activated gland. The high BMR reached by the second week of treatment was maintained. Four of the 43 animals became refractory as revealed by a return of BMR to normal. Two of the 4 were autopsied and had thyroids with normal iodine content and structure. Of the remaining 26 guinea pigs, treatment in 19 was terminated between the 38th and 80th day. Fourteen showed a continued activation and 5 had become refractory. Treatment of the remaining 7 animals was terminated in 80-88 days. Six of these showed continued thyroid stimulation and one had developed a refractory condition.[‡]

C. *Controls.* There were 21 controls, 14 of which were injected daily with a flavianate preparation made from beef muscle and 7 were untreated. No difference between the 2 groups was found in the BMR or in the structure or iodine content of their thyroids, all being within the normal range.

A marked difference in the development of a refractory condition of the thyroid in prolonged administration of 2 different preparations made from the hypophyses of the beef has thus been found. The potency of these 2 preparations was nearly identical. These findings strongly indicate that the development of a refractory condition in prolonged treatments with preparations containing the thyrotropic principle is not due to an exhaustion of the thyroid or to the formation of an "antihormone."

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[3] Friedgood, H. B., *B.J.H.H.*, 1934, **54**, 48.

[‡] Two rabbits treated 59 days with the flavianic preparation showed marked thyroid activation structurally and a very low gland iodine content.

In 3 animals not included above, treated for 47, 60 and 85 days, there was a mild elevation of BMR and moderate gland activation but the iodine values were a low normal. It is felt that these animals were becoming refractory, with a return of the gland iodine to normal preceding the microscopic change.

8635 P

Antibody Nature of Refractoriness to Injections of Hypophyseal Extracts Containing Thyrotropic Hormone.*

SIDNEY C. WERNER. (Introduced by P. E. Smith.)

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It has been shown in the preceding paper that the development of refractoriness to repeated injections of thyrotropic hormone is influenced by the type of extract in which the hormone is contained. This suggests that refractoriness is an immune response to foreign proteins and is not due to the formation of an "antihormone." If this is the explanation, preparations not inducing refractoriness would be less antigenic. This decrease in antigenicity has been tested and confirmed. No gland iodine determinations were done in this series. The extracts and methods used were the same as in the preceding paper.

A. *Effect of non-stimulating doses of the* Na_2SO_4 *and the flavianate preparations of beef anterior hypophyses on the development of refractoriness in the thyroid.* Two series of 6 male guinea pigs each, weighing 250-350 gm., were used. Five of the first series were given 0.1 cc. of the Na_2SO_4 preparation subcutaneously every other day for 32 days. They showed no rise in the BMR. Twenty times this amount (2 cc.) then given daily for 5 days caused no activation of the thyroid, as revealed by the BMR and biopsy specimens of thyroid. The previously untreated control showed a marked response to this large dosage. The second series was treated like the above except that the flavianate extract was used and the treatment lasted for 41 days (0.02 gm. dried gland equivalent was given at each injection). No elevation of BMR took place during this treatment. Twenty times the initial

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* Aided by a Grant, administered by Dr. P. E. Smith, from the Committee for Research on Problems of Sex, The National Research Council.

dosage (0.4 gm. dried gland equivalent) was then injected daily for 4 days. It caused marked thyroid stimulation in 3 of the 5 treated animals as well as in the control. The other 2 animals showed only slight thyroid stimulation.

B. *Absence of inhibitory substances in serum of animals treated with the flavianate preparation of beef anterior hypophysis.* Three rabbits and 2 sheep were used. One rabbit received daily intraperitoneal injections of one cc. of the Na_2SO_4 preparation for 30 days and 2 rabbits 0.2 gm. equivalent of dried beef hypophysis as the flavianate daily for 50 days. One sheep was given 0.4 gm. dried gland equivalent of the flavianate intraperitoneally each day for 45 days and then 1.6 gm. equivalent for 46 days more. The other sheep was treated daily with the equivalent of 1.6 gm. dried hypophysis as a flavianate for 41 days. No inhibitory properties against the flavianate extract were found in the serum of either the sheep or the rabbits using the technic of Collip *et al.*[1] of injection of the serum and the extract into normal guinea pigs.

C. *Renewed thyroid stimulation by the flavianate preparation in guinea pigs refractory to the Na_2SO_4 preparation.* Fourteen guinea pigs were made refractory to the Na_2SO_4 preparation of beef hypophysis by daily injections for 35-45 days. Ten of these received one cc. daily and 4 received 0.1 cc. every other day. To test for refractoriness 2 cc. of the same preparation was then given daily for 4 days. No response to this increased dosage took place as revealed by the BMR and small thyroid biopsy specimens. Eleven of these animals were then injected daily with 0.5 gm. equivalent of the beef hypophyseal flavianate extract for 4 days, a dosage about equal in potency to 2 cc. of the Na_2SO_4 preparation. Definite elevation of BMR and activation of the thyroid gland structurally occurred in 9 of the 11 animals. Two were not stimulated. As controls, the other 3 guinea pigs were continued on the 2 cc. dose of the Na_2SO_4 preparation for 4 days after biopsy. These showed no thyroid activation or rise in the BMR at the termination of the treatment.

Four points indicate that as regards the thyrotropic principle the alleged "antihormone" formation is an immune response to proteins associated with the hormone. First, the development of the refractoriness depends on the type and not the potency of the thyrotropic preparation. Second, immunizing but non-stimulating doses of a certain preparation produce refractoriness as efficiently as do large doses, whereas another preparation—the flavianate—only incompletely immunizes in small doses. Third, the sera of animals chronically treated with the flavianate preparation show no inhibitory properties against it. Fourth, the thyroids of animals which have become refractory to the Na_2SO_4 preparation are stimulated when the flavianate preparation is injected even though the extract is made from anterior hypophyses of the same species of animal—the ox.

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[1] Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, **1**, 28; Collip, J. B. and Anderson, E. M., *Lancet*, 1934, **1**, 76; Bachman, C., Collip, J. B. and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 544.

The flavianate has some degree of antigenicity, as shown by partial immunization and by its partial interaction with antibodies against the other preparation used. This remaining antigenicity suggests that a species which forms antibodies more readily than the guinea pig, might become immunized to the flavianate extract and hence refractory to its injection. This is indicated by the work of Evans *et al.*[2]

8636 P

Cytology of the Monkey Endometrium Under Influence of Follicular and Corpus Luteum Hormones.*

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The histologic features of the endometrium of *Macacus rhesus* monkeys under treatment with the ovarian hormones have already been described by one of us (Hisaw[1]). We have had the opportunity to make cytological studies involving the use of various special stains upon specimens from 4 uteri under the influence of the ovarian follicular hormone (oestrin) and upon specimens from 6 uteri under the influence of the corpus luteum hormone (corporin). The monkeys used were castrated immature or young adult females, and the dosage and duration of treatment were sufficient to produce the histologic pictures in the endometrium which have been described (Hisaw[1]) as characteristic of oestrin or of corporin stimulation.

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[2] Evans, H. M., Pencharz, R. I. and Simpson, M. E., *Endoc.*, 1935, **19**, 509.

* Aided in part by the National Research Council, Committee on Problems of Sex, and in part by the Wisconsin Alumni Research Foundation.

[1] Hisaw, F. L., *Am. J. Ob. and Gyn.*, 1935, **29**, 638.

Glycogen. Special staining methods do not ordinarily reveal the presence of any glycogen in the endometrial epithelial cells during treatment with pure oestrin. However, minute traces of glycogen may be found occasionally in the basal ends of some cells following prolonged treatment with large doses of oestrin. The presence of glycogen is a characteristic feature of the endometrial response to the administration of corporin. One of the earliest manifestations of corporin influence, as revealed by ordinary routine staining methods, is a migration of the epithelial nuclei toward the lumina of the glands, leaving a conspicuous clear zone between the nuclei and the basement membrane (Hisaw[1]). Special stains reveal that this clear zone contains masses of glycogen which form beneath the nuclei and separate them from the basement membrane. With continued corporin administration the nuclei return after a few days to the basement membrane, and the glycogen masses are found at the luminal ends of the cells. During the transitional period glycogen is found at both poles of many cells, and small particles of glycogen may be seen lying along the sides of the nuclei. This appearance suggests that the glycogen migrates from the basal to the luminal end of the cells in particulate form.

The accumulated glycogen masses enlarge the luminal ends of the cells into club-like protrusions. This phenomenon produces the frayed appearance of the luminal ends of the cells which is seen with ordinary staining methods. The glycogen escapes from the cells into the lumina of the glands in masses varying from 1 to 10 micra in diameter. When corporin treatment is much prolonged a condition of secretory exhaustion results, in which most of the glycogen is found in the lumina of the glands, and very little remains in the shrunken epithelial cells.

The Golgi Apparatus. Appropriate stains reveal the Golgi apparatus as a conspicuous skein lying between the nucleus and the lumen in the cells of the endometrial epithelium. Inasmuch as the Golgi apparatus is alleged to have an important function in the process of secretion, one might expect it to present conspicuous modifications in the course of the cyclic changes in the endometrium. In this expectation, however, we have been disappointed. Under the influence of corporin the Golgi skein becomes somewhat larger and coarser than in the oestrin type of endometrium, but no other difference has been observed.

Mucin. We have not found mucin in the endometrium of untreated castrated monkeys, or of monkeys under treatment with oestrin. Mucin appears much later than glycogen following corporin administration. The mucin which appears in the endometrium under corporin influence presents 2 very striking differences from gastrointestinal mucin when stained with the standard Mayer's mucicarmine method. Whereas mucin may be stained in the form of large globules within such cells as the intestinal goblet cells, we have never been able to obtain a staining reaction for mucin in the epithelial cells of the endometrium, but have found it only in the lumina of the glands. Moreover gastrointestinal mucin stains deep red with mucicarmine, while the mucin which is seen in the endometrial glands stains only a faint pink color.

Fat. The Scharlach R stain did not reveal the presence of fat in the uninjured endometrial stroma or epithelium, or in the secretion either in the castrated monkey or in animals under treatment with oestrin or corporin. Following trauma of the endometrium, as a result of biopsy, fat is usually present in the interstitial cells as well as in phagocytes.

Epithelial Proliferations. One of us (Hisaw[1]) has called attention to a very peculiar type of epithelial proliferation which occurs following operative trauma of the endometrium in the presence of corporin stimulation. These nests of epithelial cells present a striking likeness to the epithelial proliferations which Hartman and Streeter[2] have found at the implantation site of an ovum in the monkey. In our material mucin and fat were completely absent from these cells. Most of the cells also contained no glycogen though certain scattered epithelioid cells and nests of cells exhibited a conspicuous layer of glycogen surrounding the nuclei.

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[2] Hartman, C. G. and Streeter, G. L., personal communication.